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MULTIVARIATE GEOGRAPHICAL VARIATION IN THE WOLF *CANIS LUPUS* L.[1]

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INTRODUCTION

Modern biology is subdivided in numerous disciplines dealing with particular aspects of life. But what lives and evolves in reality is not isolated characteristics, it is whole organisms or, even more exactly, populations of organisms. Nowhere else perhaps as much as in evolutionary studies has a synthetic approach become important. While this makes the theory of evolution the focus of biology, it also creates intricate problems. The student of evolution often has to compare groups of organisms with respect to multiple characters. Multivariate statistical techniques should be most efficient in such problems but comparatively little use has been made of them. The following analysis of geographical variation in the wolf (*Canis lupus* L.) is an attempt to evaluate these techniques in practice. Although only morphological characters are considered here, physiological, behavioral and ecological data could be analysed in the same manner.

The last comprehensive taxonomic study of North American wolves was that of Goldman (Young and Goldman, 1944). It consisted primarily of qualitative skull and pelage descriptions and failed to show clearly the nature and the extent of geographical variation in the species as a whole. During the present study groups of specimens have been compared with respect to complexes of metrical characters. This has disclosed multivariate trends of variation between several Nearctic wolf populations. The techniques used and the results obtained are exposed and discussed in this paper.

MATERIAL AND DATA

Numerous wolf specimens were collected in northwestern Canada by the Canadian Wildlife Service and the Manitoba Game Department during recent predator control operations. This material was deposited in the Museum of Zoology of the University of British Columbia. In this study it has been compared with British Columbia, Alaska and Arctic material, some of which was bor-

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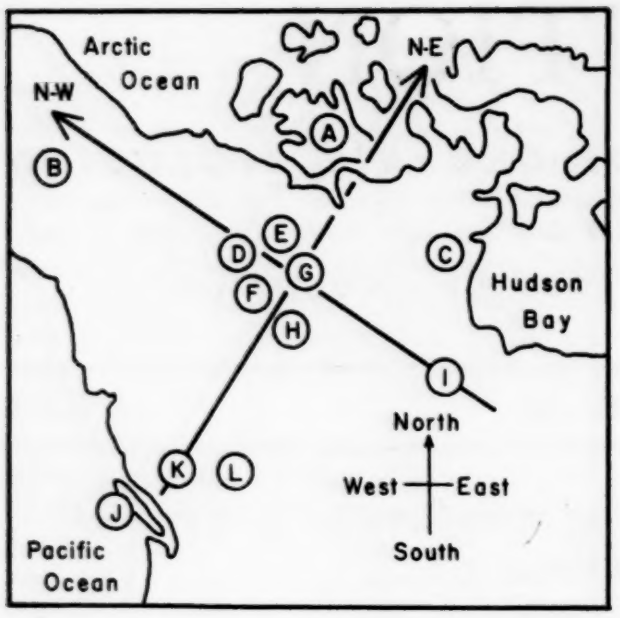

FIG. 1. Geographical origin of the samples.

rowed from the British Columbia Provincial Museum, the National Museum of Canada and Dr. R. Rausch, Anchorage, Alaska. Approximately five hundred specimens were studied. They were grouped according to sex and geographical origin as shown in figure 1 and table 1. Juvenile specimens were excluded as there was only half a dozen of them. Four general areas are represented by large samples: British Columbia (group K), Manitoba (I), and the Northwest Territories between Great Slave Lake and Great Bear Lake (groups D + E and group G). Two arrows have been lined up on these large samples in the map (fig. 1) and in a subsequent graph (fig. 10). They point approximately northeastward and northwestward and help to refer biometrical differences to their geographical context.

Skulls were available for most specimens while there were pelage and body data for only part of the material. The analysis of geographical variation was therefore based primarily on skull dimensions. Photographic transparencies of the carcasses were available for four samples of the Northwest Territories and they were examined with respect to pelage coloration. Twelve skull dimensions were measured, in the manner illustrated (fig. 2). They are referred to hereafter by the following coded designations:

L_1: Condylobasal length
L_2: Palatal length
L_3: Postpalatal length
W_1: Zygomatic width
W_2: Palatal width outside the first upper molars M_1
W_3: Palatal width inside the second upper premolars Pm_2
W_4: Width between the postglenoid foramina
W_5: Interorbital width
C_1: Least width of the braincase
C_2: Width between the auditory bullae
T_1: Alveolar length of the upper carnassial Pm_4
T_2: Crown length of the first upper molar M_1

TECHNIQUES OF ANALYSIS

The advantages of multivariate statistical techniques for evolutionary problems have been discussed by Anderson (1954). Typically evolutionary studies lead to comparisons of groups of organisms with respect to numerous charac-

TABLE 1. *Size and sex-composition of the samples*

Locality	Group	Males	Females	Undetermined	Total
Arctic	A	11	8	—	19
Alaska	B	3	3	3	9
Keewatin	C	5	3	6	14
Northwest	D	41	39	—	80
Territories	E	41	40	—	81
	F	12	8	—	20
	G	33	33	—	66
	H	—	—	9	9
Manitoba	I	73	64	—	137
Vancouver Island	J	5	5	—	10
Mainland of B. C.	K	15	12	18	45
Rocky Mountains	L	6	3	—	9
					499

teristics. But in reality, as stressed by Olson and Miller (1958), the various aspects of living organisms distinguished by man are intimately associated. When comparing evolutionary groups of organisms it is therefore best to consider as many characters as possible simultaneously. Theoretically such joint comparisons call for multivariate analysis.

Practical reasons as well as theoretical ones make multivariate techniques desirable in evolutionary studies. Groups of organisms may be entirely distinct with respect to several characters jointly and yet overlap with respect to every one of the same characters separately. Hypothetical examples of such cases are illustrated here. In figure 3 for instance two groups of specimens, represented respectively by open circles and solid dots, occupy readily separable portions of a bivariate scatter diagram. But the complete separation of these two sam-

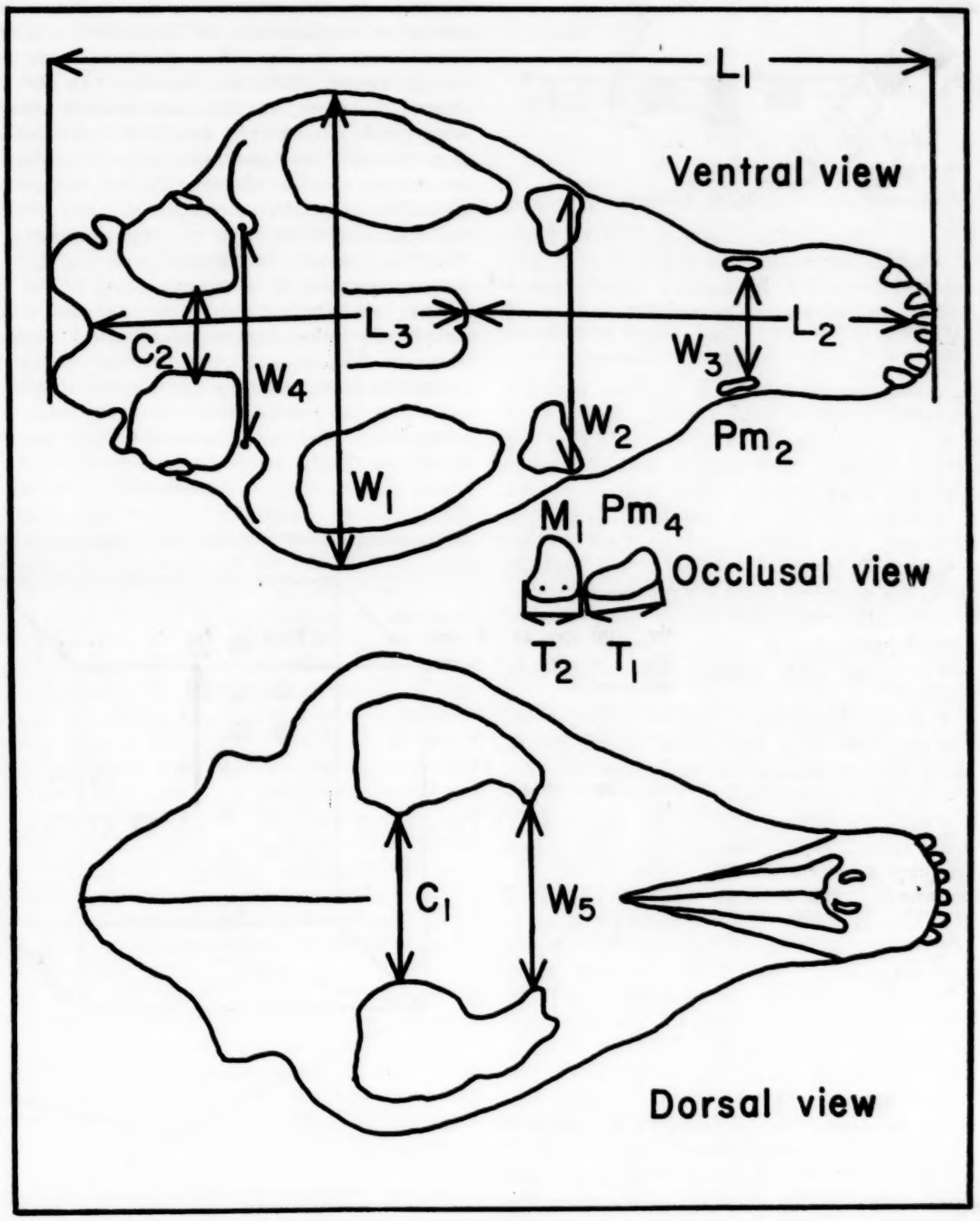

FIG. 2. Skull dimensions measured and coded designations; dorsal and ventral views of the skull and occlusal view of left upper teeth.

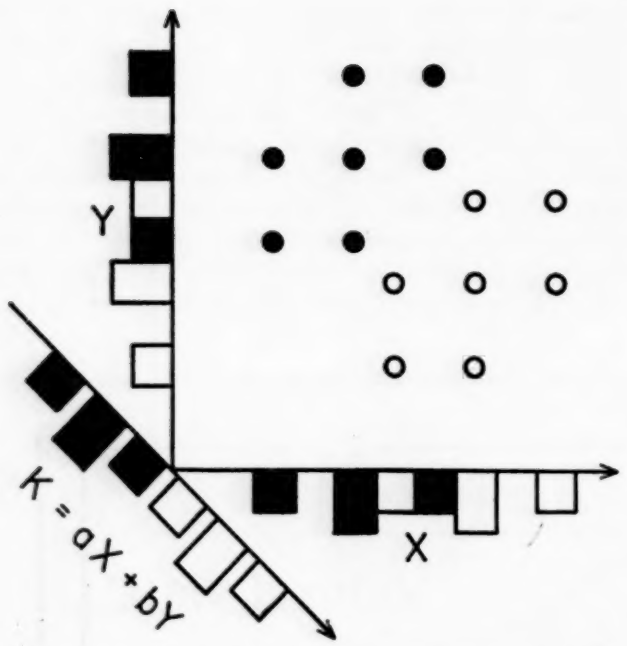

FIG. 3. Hypothetical example of discriminatory analysis for two characters. Scatter diagram and frequency histograms of characters X and Y and of discriminant function $K = aX + bY$.

ples would fail to appear if the two metrical characters X and Y were considered one after the other. This can be seen by projecting the bivariate diagram upon axes X and Y respectively; the black and the white frequency bars are interspersed in the resulting frequency histograms. The difference of the two samples could be fully expressed by a single variable though, the so-called *discriminant function* $K = aX + bY$; it corresponds to the projection of the graph upon axis K and its frequency histograms of the two samples are completely separated.

For three characters a scatter diagram assumes the form of a box within which groups of dots, circles and crosses are dispersed (fig. 4). The three edges OX, OY and OZ of the box are the coordinate axes of characters X, Y and Z. Bivariate diagrams of these data would correspond to perpendicular views of the tridimensional graph through faces XOY, XOZ and YOZ. However the true nature and magnitude of the differences between the groups would not show up well if the characters were considered one by one or two by two. The relative position of the samples appears much more clearly if the graph is viewed through the discriminant plane K_1OK_2 or projected upon it. This optimum two-dimensional representation of between-group differences is obtained by calculating the discriminant functions $K_1 = a_1X + b_1Y + c_1Z$ and $K_2 = a_2X + b_2Y + c_2Z$ and making their scatter diagram (fig. 5). The projections X, Y and Z of coordinate axes OX, OY and OZ upon the discriminant plane (fig. 5) indicate its signification in terms of the original variables themselves, the three metrical characters.

But it is in joint studies of four or more characters that the efficiency of discriminant functions is unequalled for ordinary graphing methods are impossible in more than three dimensions. Nevertheless multivariate procedures permit the analyst to examine optimum two-dimensional slices of the unexisting multidimensional graph (fig. 10, 11, 12). Much time can also thus be saved; no lengthy univariate or bivariate search is necessary for the most informative combinations of characters. There would be sixty-six different manners to associate twelve characters two by two for instance; but one or two discriminant graphs bring out almost all available information. Discriminant functions then are indispensable not only to disclose the true degree of distinctness of multivariate samples but also to condense the information relative to large numbers of characters in very few graphs.

Many samples of biometrical data follow the normal distribution at least approximately. Graphically they appear as elliptical clusters of points (fig. 8). In practice much of the information contained by such samples should be explicit in the scatter diagrams themselves. But comparisons involving several large samples would be likely to engender confusion. To avoid this, statistical summarization is necessary. *Equal frequency ellipses* are particularly effective for normal bivariate data; they

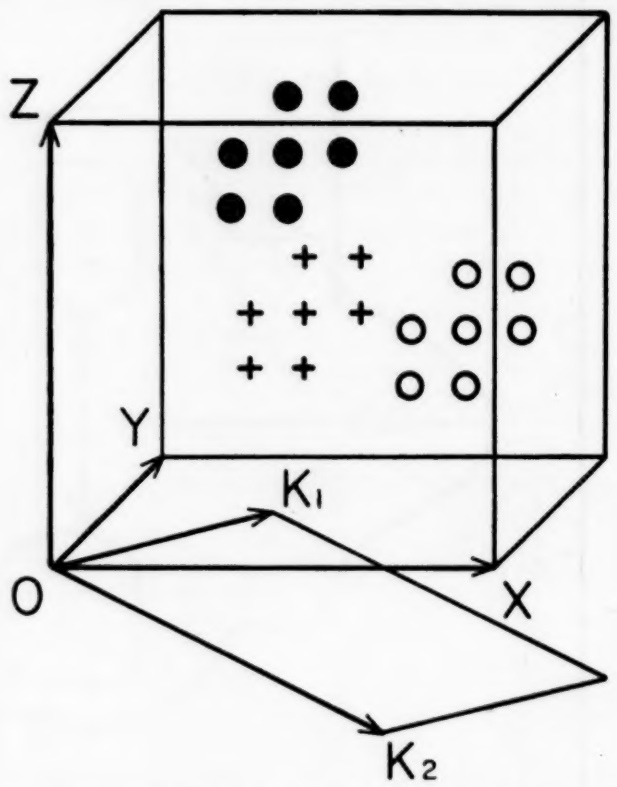

FIG. 4. Hypothetical example of discriminatory analysis for three characters. Scatter diagram of characters X, Y and Z and discriminant plane K_1OK_2 (see fig. 5).

have been used for instance by Kotaka (1953) on *Anadara granosa* (Pleistocene pelecypod from Japan). Their biometrical utilization has been thoroughly discussed by Defrise-Gussenhoven (1955). Confidence belts about regression lines and quadrilateral range diagrams have also been used to represent bivariate dispersion. However a regression line yields an estimate of the value of one character from others and this is not of primary importance in studies of evolution. As for the range diagrams utilized by Imbrie in a study of Triassic amphibians (Colbert and Imbrie, 1956), they would be preferable to ellipses only for certain abnormal types of data; but departures from normality are often negligible in practice and, when important, they can frequently be corrected by using logarithmic instead of arithmetic values. In general then equal frequency ellipses are more suitable than any other current statistical device to outline the range of variation of bivariate specimens. And, used in conjunction with discriminant scatter diagrams, they are applicable to studies of any number of characters.

Multivariate calculations follow the same basic plan however many variables are analyzed jointly. The average value of metrical characters in a group of specimens is the mean; it is the center of the sample of points. The variation of the characters within this group of organisms is summarized numerically by a set of mean squared deviations and cross products, the so-called *within-group covariance matrix* W. Matrix W is generated by the elliptical dispersion of the individuals around their group mean and it figures in the equation of the equal frequency ellipses (or multidimensional ellipsoids) circumscribing the sample. The multivariate analysis of within-group variation consists in calculating the *principal axes* of such ellipses; to these directions of maximum variation (or *characteristic vectors*) correspond *principal variance components* (or *characteristic roots*). It is when biometrical samples are examined with respect to these principal trends of variation that their information content is the most explicit. Principal component analysis is closely similar in principle to factor analysis as applied by psychologists and, more recently, by biometricians.

As noted by Yates (1950), the analysis of differences between groups of specimens is analogous to that of within-group variation. The dispersion of group means around their grand mean is expressed by the *between-group covariance matrix* B. The so-called *discriminant functions* are the principal axes of matrix B after standardization by matrix W of within-group variation (pooled over all samples). Geometrically speaking the ellipsoid of within-group variation is taken as a yardstick for between-group variation. But, within ordinary

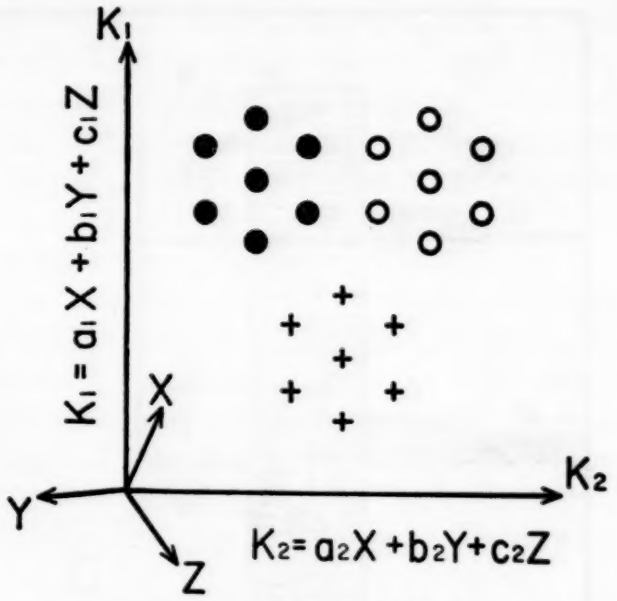

FIG. 5. Scatter diagram of discriminant functions K_1 and K_2 corresponding to plane K_1OK_2 of figure 4 and projections thereupon of axes OX, OY and OZ.

samples of living organisms, the greatest variation component of body dimensions is generally age and size (Teissier, 1955; Wright, 1932). Consequently the standardization of matrix B by matrix W emphasizes other factors of variation than age and size. The purpose formerly served by age classes and size-independent ratios is thus automatically fulfilled in discriminatory analysis. In multivariate group comparisons as in principal component analysis it is in the direction of the principal axes that the observations differ the most and that most of the information lies.

Several expositions of multivariate methods are now available (Hotelling, 1954; Kendall, 1957; Quenouille, 1952; Rao, 1952); to understand them elements of multidimensional algebra and geometry are indispensable; Murdoch's excellent introduction (1957) provides a relatively easy way to the latter. Most recent applications of multivariate analysis have unfortunately been too abstract; the graphical means of presentation utilized in this study make multivariate results just as easy to visualize as univariate or bivariate ones. As for the length of computations, it is no longer prohibitive thanks to the growing availability of electronic digital computers. Multivariate statistical techniques should be used more and more along with simpler methods when problems call for them. This appears to be the case in biometrical studies of evolution.

PELAGE COLOR VARIATION

The pelage coloration of wolves is very variable in intensity, in hue and in pattern. As detailed verbal descriptions are not

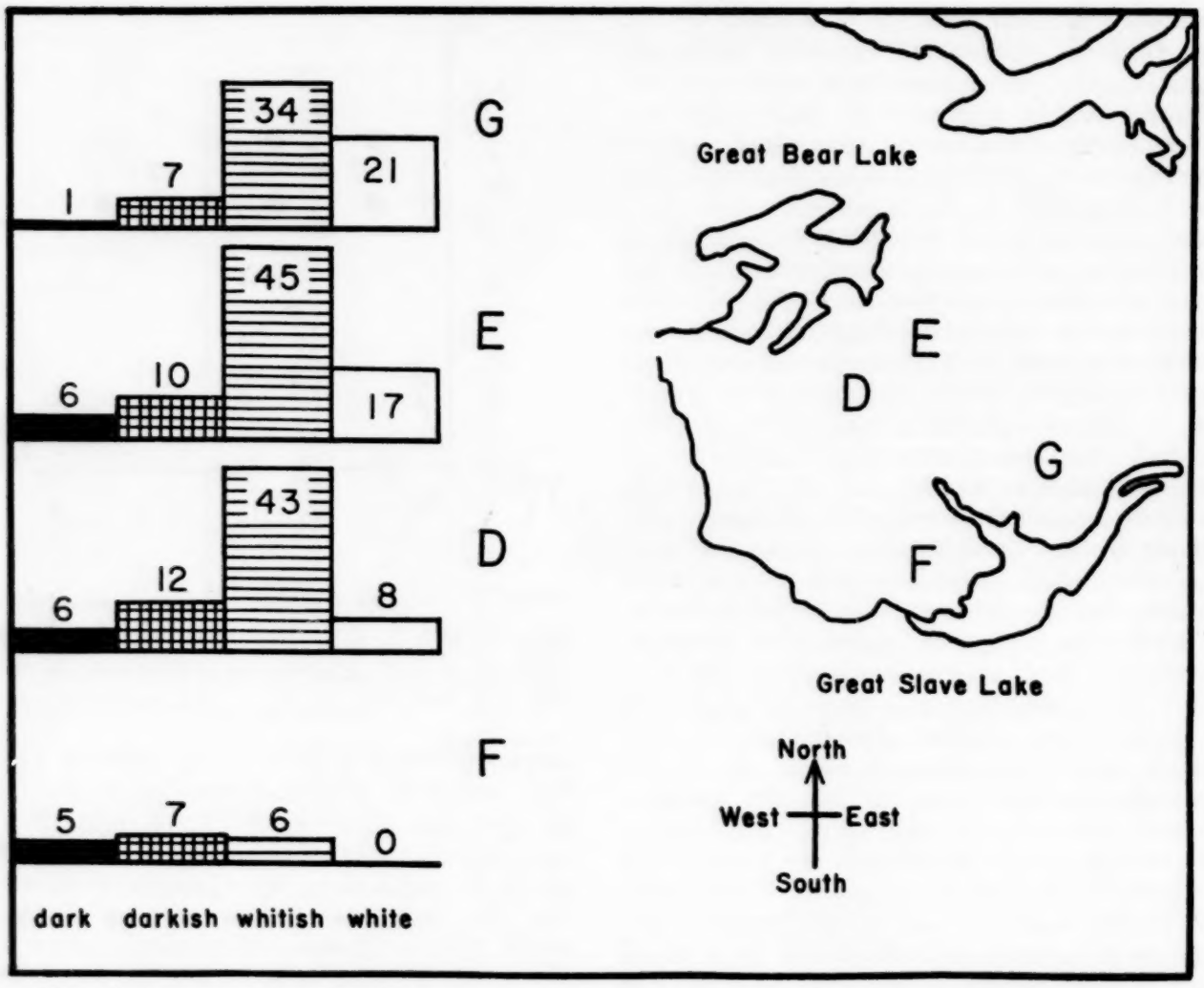

FIG. 6. Northeastward increase in the relative frequency of pale wolves toward the tundra between Great Slave Lake and Great Bear Lake.

suitable for large samples, the photographic transparencies examined were classified into four arbitrary classes according to the general darkness of pigmentation: dark, darkish, whitish and white. Such arbitrary classes do not correspond in the wolf to actually discrete color phases as in some polymorphic species. Such a classification is also only approximate and fits adequately only the present material. It does disclose however gradual differences in color-class frequencies analogous to the clines in color-phase frequencies of the red fox and the black bear (Cowan, 1938).

The relative frequency of pale wolves increases in a northeastward direction (toward the tundra) between Great Slave Lake and Great Bear Lake in the Northwest Territories (fig. 6). There are gradually more white and whitish and fewer dark and darkish individuals in samples F, D, E and G successively. Samples D and E differ little from each other but differ significantly from the two extreme groups (95% chi-square). Seasonal variation is apparently not involved since specimens were collected at comparable dates. On the other hand, the environmental conditions of these four localities appear too similar for such pronounced differences to be phenotypical. Consequently the color frequency shift probably expresses a cline in gene frequencies. The extreme whiteness of tundra in winter is of course very well known and the higher frequency of pale wolves there is clearly a case of homochromy. The selective value of concealing coloration for a predator would pre-

sumably lie in the corresponding ease to approach preys. Pale pelage coloration may be just one facet of a physiological complex however and it is possibly related to other factors than homochromy.

Recent barren-ground caribou studies (Banfield, 1954; Kelsall, 1957) have shown caribou to migrate more intensely through areas D and E than through areas F and G. The similarity of pelage coloration between wolf populations appears to be proportional thus to the local intensity of caribou migrations. This relationship could be expected if the movements of wolves were correlated with those of caribou. The latter correlation is effectively suggested by field observations. Wolves are often seen with caribou herds (Rausch, 1951) and, statistically speaking at least, they probably follow them at migration (Banfield, 1951).

A relatively higher frequency of dark individuals has been reported in the Rocky Mountains (Cowan, 1947). The short-distance cline exhibited by the present material may therefore be part of a long-distance cline going at least from the Rockies to the Northwest Territories.

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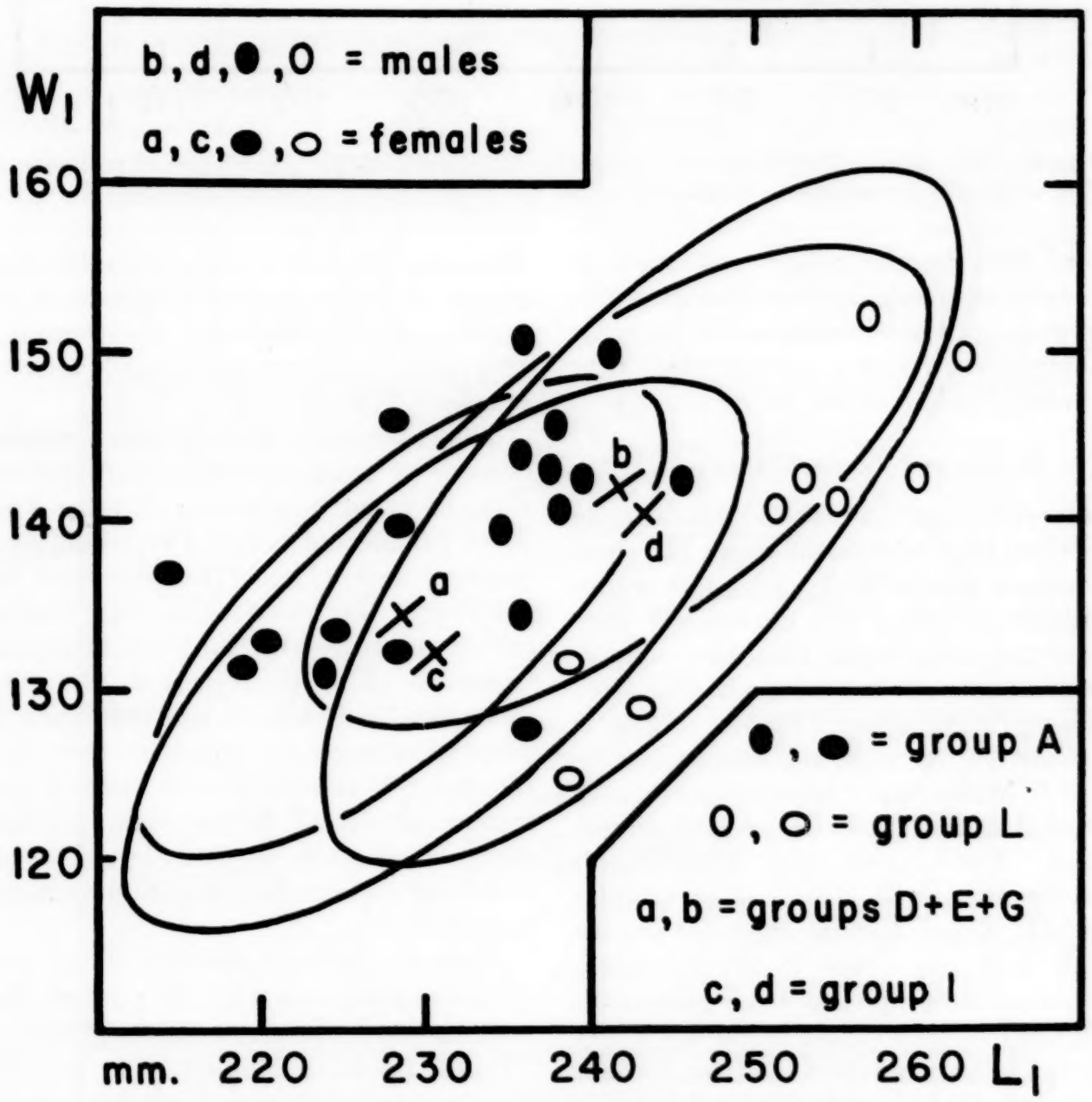

FIG. 7. Condylobasal length (L_1) and zygomatic width (W_1). Dots represent individual specimens; crosses and ellipses represent means and 95% frequency intervals respectively. Males have a larger skull than females and northeastern individuals are shorter and relatively broader-skulled than southwestern ones.

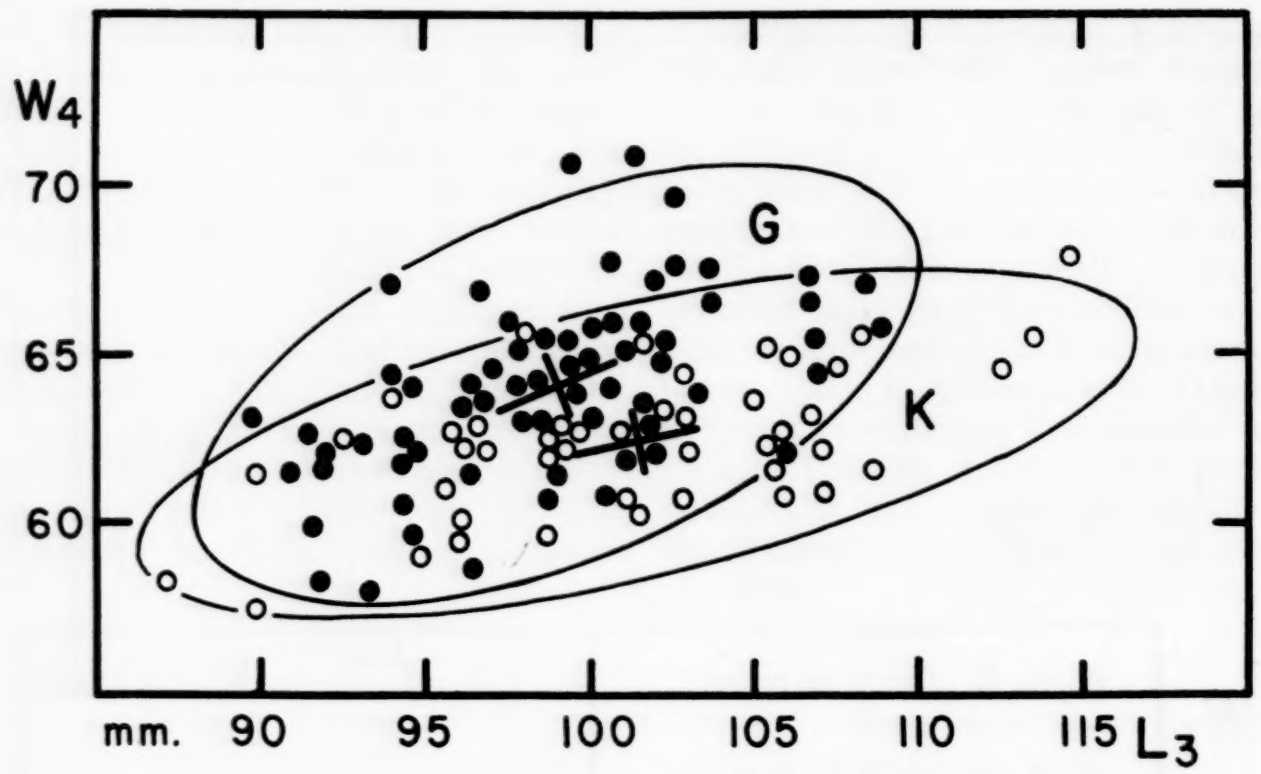

FIG. 8. The wolves of the Northwest Territories (G) have a higher growth rate of postglenoid width (W_4) relatively to postpalatal length (L_3) than those of British Columbia (K).

More data on the pelage coloration of wolves would perhaps show analogies with the geographical distribution of the color phases of the red fox and the black bear (Butler, 1947; Cowan, 1938).

BIVARIATE SKULL VARIATION

Overall skull size can be satisfactorily described by condylobasal length (L_1) and zygomatic width (W_1). Bivariate scatter diagrams of these two dimensions were made and 95% equal frequency ellipses were calculated following Defrise-Gussenhoven's procedure (1955). Figure 7 summarizes the most important information. Males are approximately 4% larger than females in linear skull dimensions. This agrees with Hildebrand's conclusions on the body size of Canidae (1952). Other facts brought out are the small skull size (close to the left lower corner of the graph) and the great relative skull breadth (close to the left upper corner) of northeastern wolves. Groups L, I, D + E + G, and A are successively closer to the left side of the graph. This ordering of samples according to skull size and relative breadth is strikingly similar to the ordering of the geographical localities of origin projected upon a line of northeastward direction. Such gradual geographic variation was termed *clines* by Huxley (1938).

The shortness and greater relative breadth of skull of northeastern wolves also shows up in a scatter diagram (fig. 8) of postglenoid breath (W_4) and postpalatal length (L_3). The wolves of the Northwest Territories (G) are shorter and broader-skulled than those of British Columbia (K) with respect to these two dimensions. But here the difference of proportion increases with size; there is a difference of relative growth rate. Equal frequency ellipses fit the data satisfactorily; there is no obvious curvature of trend and no need for a logarithmic transformation.

A third bivariate association shows geographical variation (fig. 9): interbullar breadth (C_2) with carnassial length (T_1). The specimens of Manitoba (I) and the Northwest Territories (D + E) are at the center of this graph and constitute the average. The wolves of British Columbia (K) have shorter upper carnassial teeth

than the average and those of Vancouver Island (J) a narrower interbullar space. Simple examination of the skulls confirms what the graphical analysis summarizes. Distinct spaces show in between the small teeth of British Columbia wolves and the ten Vancouver Island specimens have markedly inflated auditory bullae with a narrow interval. Surprisingly in this graph the Vancouver Island wolves differ the most from those to which they are the closest geographically. Further discussion of this will follow the joint multivariate analysis of all twelve skull dimensions.

Multivariate Skull Variation

Comparing biometrical samples with respect to complexes of characters requires the calculation of discriminant functions. The coefficients K of these functions and the components D of between-group variation for which they account are the solution of the following matrix equation: KB = DKW. Matrices B and W are the *between-group* and the pooled *within-group covariance matrices* respectively and their signification has already been explained in these pages. As geographical variation in skull proportions was of primary interest here, sexes were kept together; sexual skull differences were mostly size differences (fig. 7) and separating males and females here would have spread the group means too much in the direction of size. The equation KB = DKW was solved on an electronic digital computer by matrix operations (Murdoch, 1957: 165, 166) corresponding to the transformations suggested by Rao (1952: 357, 367). Matrices were diagonalized following the Jacobi method. The within-group variances and covariances of the discriminant functions were calculated to verify the computations; they were exact to two or three significant digits (KWK′ = I) and this was considered acceptable.

The sum of the components D_i of between-group variance was 75.464; of this

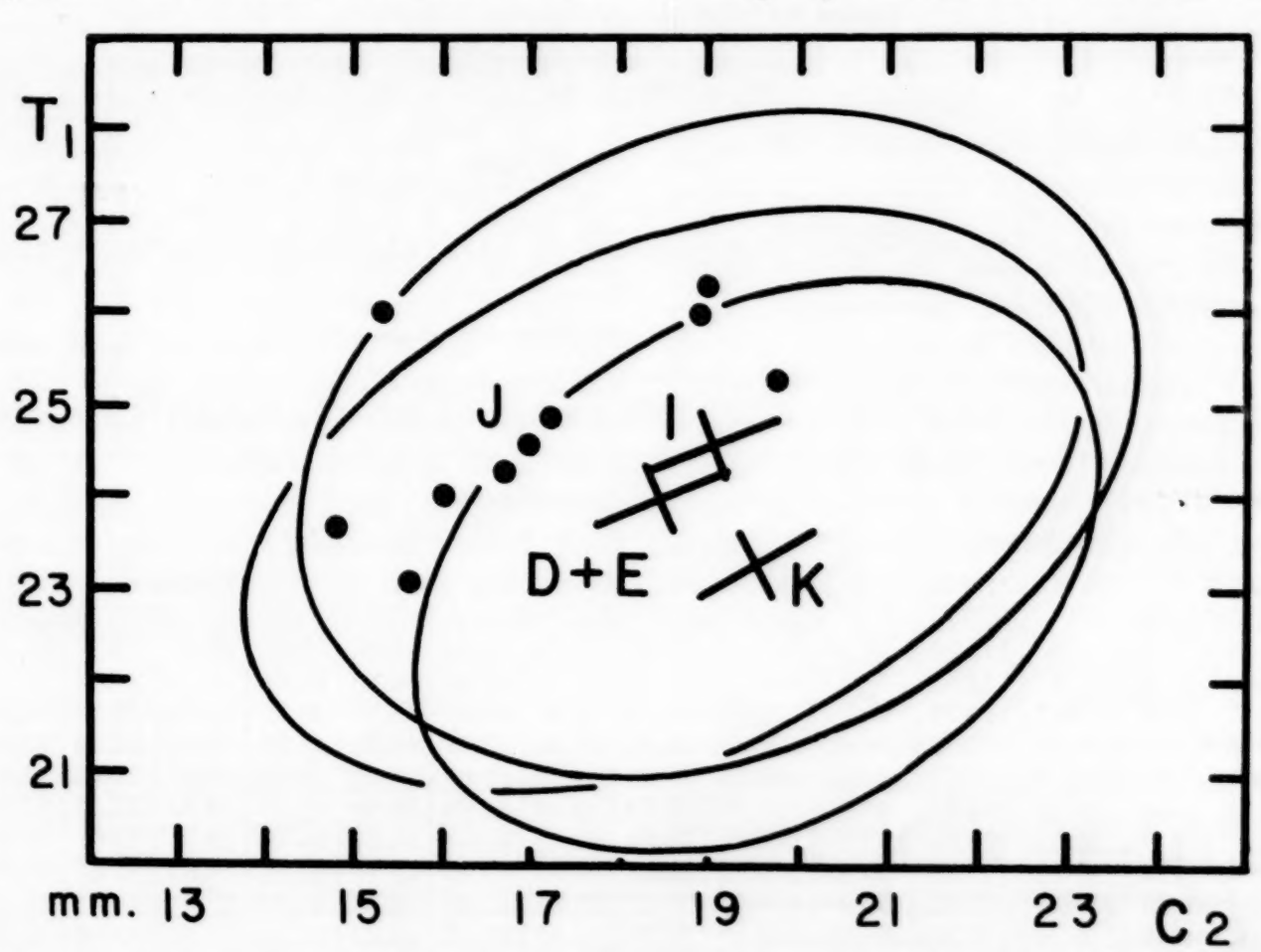

Fig. 9. The Vancouver Island specimens (J) have a narrow interbullar space (C_2) and those of British Columbia (K) short upper carnassial teeth (T_1).

TABLE 2. *Discriminatory analysis*

Variance component	D_1	D_2	D_3	D_4	D_5
Magnitude	39.274	13.474	9.008	5.958	3.206
Per cent of total	52%	18%	12%	8%	4%
Probability	<.01	<.01	<.01	<.01	<.05

94% was accounted for by the first five functions (table 2). The statistical significance of these variances was tested as prescribed by Rao (1952: 372) for large samples. If the various samples came from a same population the probability of such large components would be less than 1% for the first four and less than 5% for the fifth. To each component correspond twelve coefficients for the skull dimensions in the discriminant functions; the numerical values of these coefficients are not given here for they could be deduced from the vectors of the discriminant graphs (figs. 10 and 12). The group means, the grand mean and the pooled within-group standard deviation of the twelve skull dimensions are listed for reference (table 3); inspecting them rapidly confirms the reality of the multidimensional trends of variation disclosed by discriminatory analysis. Tabulating other statistics or raw data here would consume too much space without making anything explicit.

The pattern of dispersion of the groups in the plane of the first and second discriminant axes (fig. 10) presents similarities to the geographical disposition of the localities of origin. Northern samples tend to congregate in the left upper corner of the graph, eastern samples in the right upper corner and contrarywise for southern and western samples. The two ar-

TABLE 3. *Multivariate statistics (in mm.)*

Characters		L_1	L_2	L_3	W_1	W_2	W_3	W_4	W_5	C_1	C_2	T_1	T_2
		Grand Mean											
		236.91	118.02	99.69	137.67	78.50	33.57	63.88	45.68	41.29	18.90	24.33	17.28
		Pooled Standard Deviation											
		10.14	5.017	5.022	8.012	3.524	1.987	2.675	3.503	2.835	1.871	1.347	.8787
		Group Means											
Group	N												
A	19	231.63	113.84	98.84	139.37	80.32	32.90	65.84	45.44	40.08	19.20	25.77	17.54
B	9	245.67	123.11	102.22	140.45	81.33	35.36	65.65	46.86	43.06	19.46	24.40	17.70
C	14	234.14	116.79	98.50	135.29	78.72	33.67	64.01	45.71	40.52	18.70	24.50	17.33
D + E	161	234.73	117.30	98.49	138.56	78.37	33.58	63.78	46.37	41.28	18.45	23.99	17.28
F	20	242.15	119.55	103.15	140.75	79.63	33.79	65.07	45.64	41.64	19.67	25.13	17.42
G	66	235.98	118.35	99.00	137.76	78.13	33.18	64.05	46.08	41.03	18.59	24.42	17.36
H	9	243.33	120.89	102.89	140.22	79.59	34.39	64.34	46.27	40.49	20.54	23.91	17.19
I	137	237.20	117.75	99.88	136.52	78.63	34.01	63.82	45.11	41.15	19.03	24.61	17.34
J	10	236.30	119.60	98.30	136.70	77.73	31.85	61.15	44.13	41.94	17.03	24.82	16.70
K	45	240.18	119.36	101.40	135.27	76.92	32.97	62.49	44.07	42.16	19.50	23.28	16.80
L	9	251.00	123.45	106.33	139.67	79.91	32.73	66.10	47.51	42.61	22.23	25.23	17.76

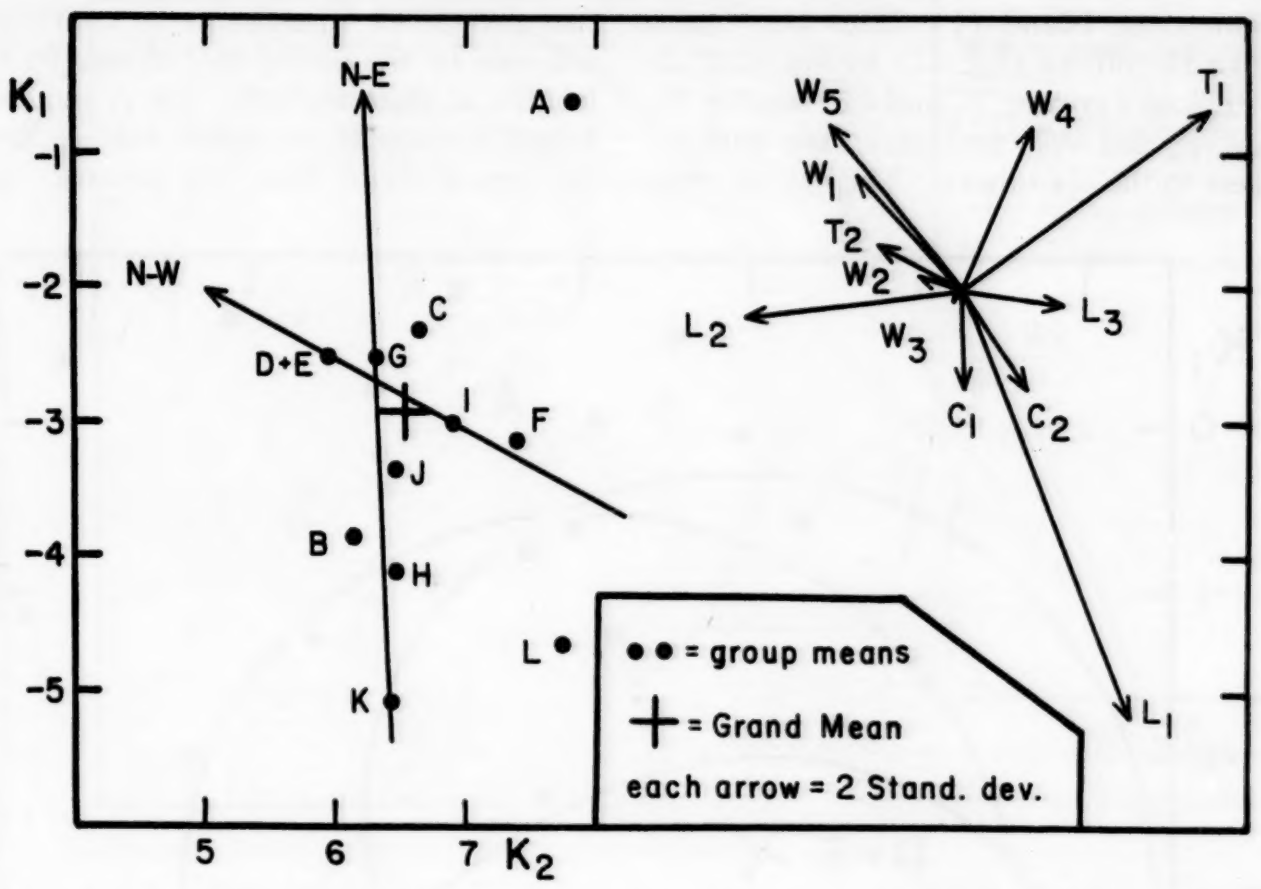

FIG. 10. Group dispersion (left) in discriminant functions K_1 and K_2 and variation of the skull dimensions (right); arrows N–E and N–W correspond to those of the map (fig. 1).

rows marked N–E and N–W correspond to those of the map (fig. 1) and help to evaluate the similarity of the pattern of biometrical dispersion to the pattern of geographical origin. Discrepancies come mostly from small samples; the most obvious one is the relative position of Alaska (B) and Vancouver Island (J) wolves. But this discrepancy is largely compensated for by the dispersion pattern of the third and fourth discriminant functions (fig. 12); here the average of the Alaska specimens diverges from the southern groups and the Vancouver Island individuals contrast sharply with all others. The first component of multivariate variance ($D_1 = 52\%$ of total), which corresponds closely to a northeastward direction, is markedly larger than the next one ($D_2 = 18\%$ of total). Geographical variation in skull dimensions would thus appear to be most pronounced northeastward. Ascertaining this last point would require more material however for the present samples are far from evenly distributed with respect to latitude and longitude.

As discussed previously, the full significance of discriminant scatter diagrams would not be explicit unless the coordinate axes of the original variables were projected thereupon. Sets of *vectors* (= arrows) bearing the coded designations of the skull dimensions indicate these projections in the discriminant graphs (figs. 10 and 12). Each vector shows the change in the discriminant functions that the corresponding dimension would generate if it varied independently. All skull dimensions are intercorrelated of course and these vectors must be considered jointly rather than separately. Northeastern wolves differ generally from southwestern ones (fig. 10) by a decrease in skull length (L_1) and in braincase development (C_1 and C_2) opposed to an increase in skull breadth (W_1, W_4 and W_5). Eastern individuals have longer upper carnassial teeth (T_1) and a shorter palate (L_2) than western ones. The wolves of

Vancouver Island (J) differ very much from the others (fig. 12) by six skull dimensions (greater T_1 and C_1; smaller T_2, C_2, W_4 and W_5) and very little with respect to the six others. The role of these two groups of dimensions is contrasted not only by the directions but also by the lengths of their vectors. The Vancouver Island specimens are much further from the grand mean than the arrows (one

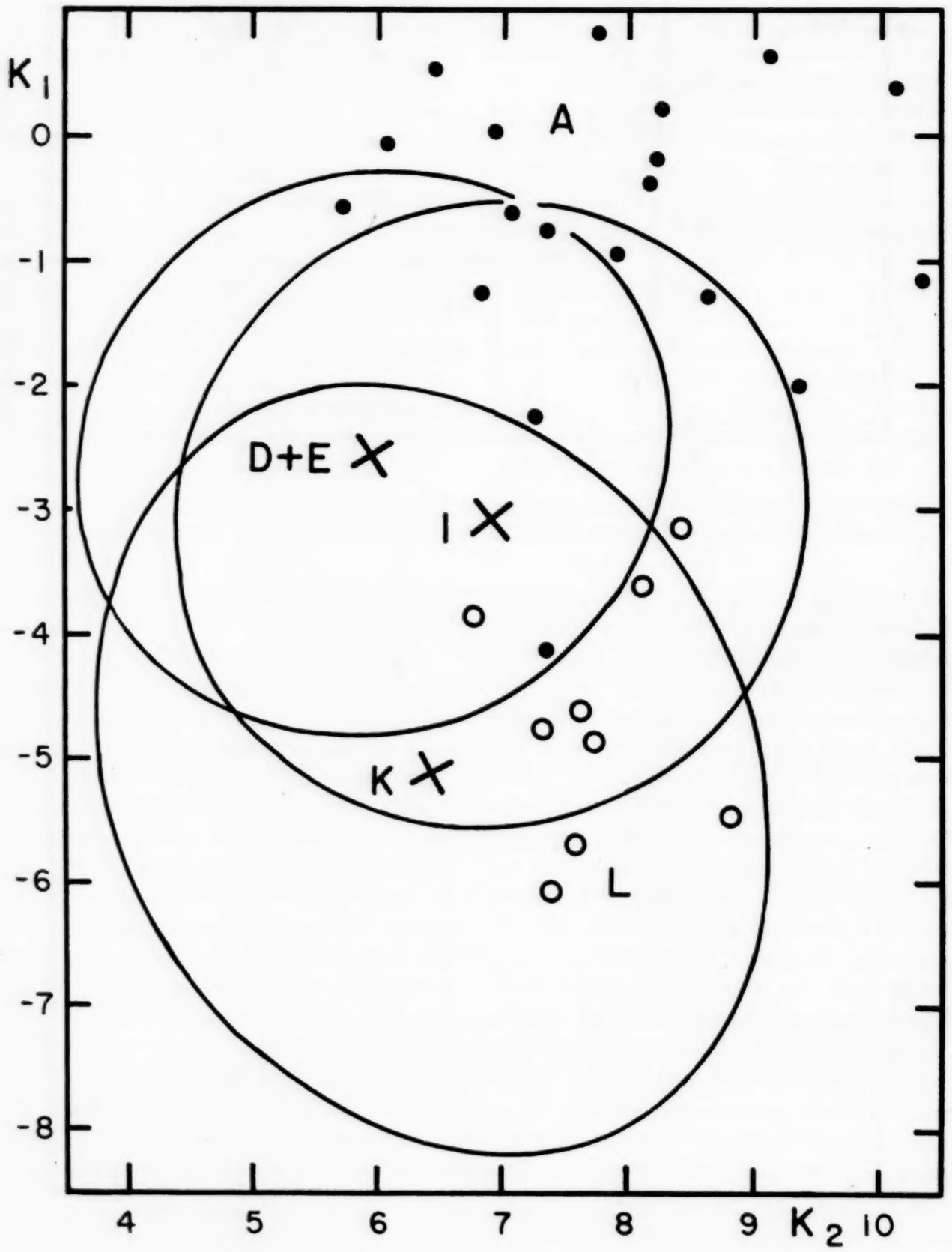

FIG. 11. Overlapping of groups in discriminant functions K_1 and K_2; dots represent individuals, mean crosses and 95% equal frequency ellipses represent samples; letters refer to closest symbols.

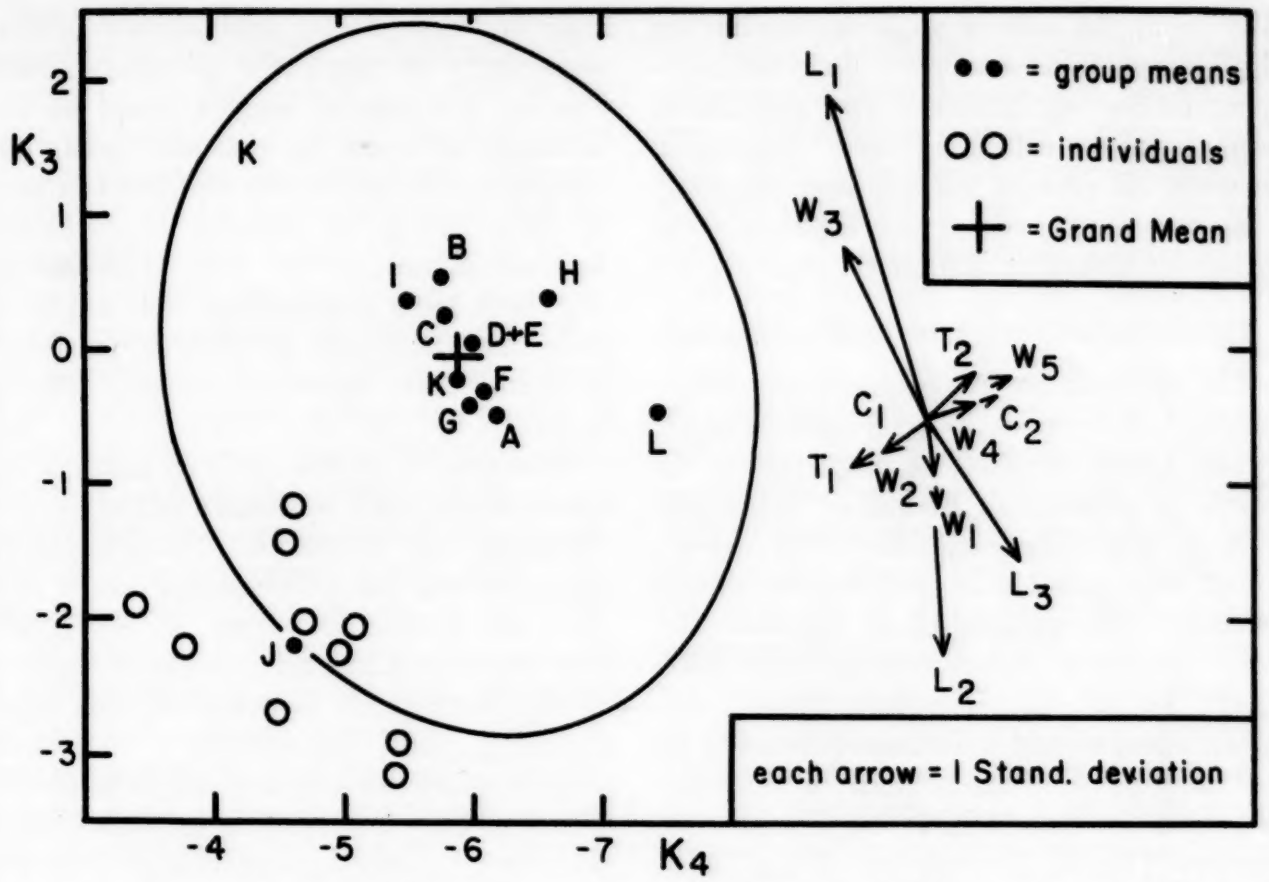

FIG. 12. Group dispersion (left) in discriminant functions K_3 and K_4 and variation of the skull dimensions (right); 95% equal frequency ellipse of group K.

standard deviation) of their discriminators are long.

The amount of overlapping of groups of specimens in discriminant graphs can be illustrated adequately with individual dots for small samples and equal-frequency ellipses for large samples. To evaluate overlapping exactly all discriminant dimensions should be considered jointly and the peripheral decrease in frequency within the samples should be taken into account. But for the sake of simplicity *biometrical overlapping* is defined here as the *percentage of common area between samples in a two-dimensional scatter diagram.* Following this definition the British Columbia wolves (K) overlap by approximately 50% (fig. 11) with those of Manitoba (I) and the Northwest Territories (D + E). The individuals of the Rocky Mountains (L) are intermediary and overlap largely both with those from British Columbia and those from Manitoba. The Arctic specimens (A) overlap by approximately 50% with those of the mainland. The lowermost point of sample A represents a subadult female from Coronation Gulf (mainland) which should apparently have been grouped with mainland specimens and is relatively narrow-skulled. This individual excepted, the Arctic wolves do not overlap with those of the Rockies. Larger samples would probably do so to some extent however. The Vancouver Island specimens (J) overlap by approximately 50% with the others (fig. 12).

To sum up, the present material shows northeastern wolves to have shorter and relatively broader skulls than southwestern ones and eastern wolves to have a shorter palate and longer upper carnassial teeth than western ones. Such a generalization is only approximate however; the pattern of biometrical differences does not correspond exactly to the pattern of geographical origin and the first two discriminant functions account for only 70% of the variance between the groups. More variance is associated with a northeastward direction than with any other one. The Vancouver Island wolves differ mark-

edly from the others with respect to six skull dimensions but very little with respect to the six others. The amount of biometrical overlapping and separation between all groups corresponds approximately to the degree of geographical separation by distance, insularity, etc.

Interpretations and Conclusions

The approximate correspondence of biometrical differences to geographical separation could readily be interpreted in terms of population genetics. Theoretically genetic differentiation within an incompletely panmictic population should increase with geographical distance and other factors of isolation (Li, 1955: 306–310). In the case of wolves, genetic differentiation should have been promoted by the immensity of their area of distribution and its subdivision by Pleistocene glaciers (Rand, 1954); but these influences should have been counteracted by the high mobility of such a large cursorial predator. The magnitude of biometrical differences between populations could be taken as indications of genetical differentiation.

The genetical interpretation of geographical variation is not the only possible one however. The specimens studied represent phenotypes and the latter have been exposed to highly varied environmental conditions. In fact environmental conditions vary throughout the area of distribution of the wolf. Arctic winters are gradually colder, darker and longer northward or northeastward for instance; and taiga is gradually replaced by tundra in a general northeastward direction. Such environmental differences might induce gradual phenotypical variation directly as well as genotypical variation indirectly. Direct effects of the environment on physiological processes and morphological characteristics are well known. Seasonal changes in temperature, illumination, humidity and food supply affect the reproductive cycles and migrations of various vertebrates. Molts and coat-color changes of weasels were controlled photoperiodically by Bissonnette and Bailey (1944), apparently through the pituitary gland. Prairie and tundra wolves could be particularly affected by seasonal periodicity as they cannot evade the climatic extremes of their habitat by seeking microclimates as small mammals do. The circumstances in which local populations live might induce modifications of growth processes, including those involved in skull development.

The growth of the various parts of the mammalian skull is neither perfectly synchronous nor isometric. The facial region reaches full development much later than the braincase (Baer, 1954). This was noted in Canidae at least as early as 1880 (Huxley). Because of this asynchronism and anisometry, temporary physiological disturbances might have very different effects according to their time of occurrence. Young northeastern wolves enter their first cold and dark arctic winter at an age (5 or 6 months) at which their facial growth potential is presumably still very high; their undersized face as adults is perhaps largely due to a metabolic and/or hormonal imbalance during this period of low temperature and illumination. Mice with the pituitary dwarf genotype differ from normal ones by *"their smaller size, their shorter snout, the short fleshy ears, and the relatively shorter tail"* (Grüneberg, 1952: 122). As for juvenile sheep with thyroid deficiencies, they grow skulls with normal braincase and teeth but with an underdeveloped facial region (Todd and Wharton, 1934); the descriptions of the latter would fit surprisingly well indeed the skulls of northeastern wolves with large teeth cramped in a short palate. The possible influence of low winter illumination on endocrine balance and growth is suggested by the effects of darkness on the teleost *Astyanax mexicanus* (Rasquin and Rosenbloom, 1954); darkness led to a loss of calcium from the bones and resulted in shorter and deeper-bodied fish than normal; skeletal changes were not repaired after return of the survivors to light.

Baer (1954) was led to postulate the existence of two major and relatively independent developmental processes in the postnatal mammalian skull; the expansion of the braincase on one hand, and the elongation of the face and the base of the skull on the other hand. It would be of great interest to see how much of variation in relative skull breadth in the present material could be ascribed to alterations of the equilibrium between two such growth processes. Very much of skull variation between the various breeds of domestic dogs (Stockard, 1941) could probably also be accounted for in this way. According to Scott's discussion (1957), the elongation of the mammalian skeleton is due to cartilage growth and promoted by pituitary growth hormones while its massiveness is due to subperiosteal growth and depends primarily on function and robustness factors. Although such a distinction seems most applicable to the postcranial skeleton, it appears to correspond also with trends of skull variation.

Whether or not the biometrical characteristics of wolves can be ascribed to specific growth processes however, the most important evolutionary problem is the extent to which such characters are genotypical or merely phenotypical. Bringing up and breeding captive animals from various localities under controlled conditions would be informative in this respect but such data are not available at present. Gradual phenotypical variation induced directly by environmental conditions is possibly present along with genotypical variation in the skull dimensions of wolves.

Taxonomical conclusions can be based on geographical variation only inasmuch as the latter is hereditary. How much of biometrical variation is inherited in the present material is unknown. Even if the biometrical differences were entirely genetical however, the overall pattern of variation between the populations sampled is more suggestive of an incompletely panmictic continuum than of distinct subspecific units. Only one population seems sharply different from its immediate neighbours, probably as a result of insular isolation, that of Vancouver Island. In fact, it resembles northern wolves more than those presently on the mainland of British Columbia. It is perhaps with northern populations that Vancouver Island had its most intense recent biotic contacts. It seems doubtful that such a small insular population would have a major long-term evolutionary significance however. Ascertaining the taxonomic relationships of northwestern Nearctic wolves would require more material from Vancouver Island, Alaska, Alberta and the regions in between. An adequate analysis of variation is of course desirable for the species as a whole or at least for all its North American representatives. There are most likely far too many subspecific designations in use (Miller and Kellog, 1955).

As far as multivariate statistical techniques are concerned, their application to the present problem has probably already made their value apparent to many readers. One last comment is made in conclusion. Discriminant scatter diagrams of contemporaneous groups of organisms are closely analogous to what sections of phylogenetic trees at given time levels should be like. Discriminatory analysis may well prove an ideal tool in phylogenetic studies. Multivariate statistical techniques may make eventually possible the exact calculation of horizontal slices of the tree of Evolution.

Summary

An analysis is made of pelage and skull variation in five hundred wolf specimens from northwestern Nearctic localities. Pale wolves are relatively more frequent toward the tundra. Males are 4% larger than females in linear skull dimensions. Northeastern specimens are shorter- but relatively broader-skulled than southwestern ones. Multivariate biometrical differences between populations appear approximately proportional to geographical separation. This may express genetic

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differentiation through imperfect panmictia. But direct environmental influences may be involved. Formal taxonomic conclusions are postponed but it seems probable that far too many subspecific designations are now in use. Multivariate statistical techniques are very efficient for evolutionary comparisons with respect to complexes of characters.

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Appendix

Multivariate Statistical Formulae[3]

Value of i^{th} character measured on an individual specimen: X_i

Set of values of the p characters measured on an individual specimen: $X = (X_1, \cdots X_p)$

Mean of a sample: $\bar{X} = (\bar{X}_1, \cdots \bar{X}_p)$

Variance of i^{th} character within a sample: W_{ii}

Covariance of i^{th} and j^{th} characters within a sample: $W_{ij} = W_{ji}$

Variance-covariance matrix of a sample:

$$W = \begin{pmatrix} W_{11} & \cdots & W_{1p} \\ \cdots & \cdots & \cdots \\ W_{p1} & \cdots & W_{pp} \end{pmatrix}$$

Equation of 95% equal frequency ellipse: $(X - \bar{X})\, W^{-1}\, (X - \bar{X})' = 5.99$

X' = X transposed; W^{-1} = inverse matrix of W; only the elements of X and W corresponding to the two characters are included here in X and W; 5.99 = 95% chi-square with 2 degrees of freedom; see Defrise-Gussenhoven (1955).

Covariance matrix of sample means around their grand mean:

$$B = \begin{pmatrix} B_{11} & \cdots & B_{1p} \\ \cdots & \cdots & \cdots \\ B_{p1} & \cdots & B_{pp} \end{pmatrix}$$

Matrix of the coefficients K_{ij} of the discriminant functions:

$$K = \begin{pmatrix} K_{11} & \cdots & K_{1p} \\ \cdots & \cdots & \cdots \\ K_{p1} & \cdots & K_{pp} \end{pmatrix}$$

Matrix of the components D_i of between-group variance corresponding to the discriminant functions:

$$D = \begin{pmatrix} D_1 & \cdots & 0 \\ \cdots & \cdots & \cdots \\ 0 & \cdots & D_p \end{pmatrix}$$

Matrix equation yielding K and D: $KB = DKW$

Coordinate of any point $X = (X_1, \cdots X_p)$ upon the i^{th} discriminant axis: $K_i = K_{i1}X_1 + \cdots + K_{ip}X_p$

Within-group variances and covariances of the discriminant functions: KWK'

K' = K transposed

$KWK' = I = \begin{pmatrix} 1 & \cdots & 0 \\ \cdots & \cdots & \cdots \\ 0 & \cdots & 1 \end{pmatrix}$ for the pooled within-group covariance matrix.

[3] A more detailed note on the calculation of discriminant functions is being prepared by S. W. Nash and P. Jolicoeur.

Literature Cited

Anderson, E. 1954. Efficient and inefficient methods of measuring specific differences. In Kempthorne and others, 1954: 93–106.

Baer, M. J. 1954. Patterns of growth of the skull as revealed by vital staining. Human Biology, **26**: 80–126.

Banfield, A. W. F. 1951. Notes on the mammals of the Mackenzie District, Northwest Territories. Arctic, **4**: 112–121.

——. 1954. Preliminary investigation of the barren ground caribou. Wildl. Man. Bul. 1, 10 A and 10 B. Canadian Wildlife Service, Ottawa.

Bissonnette, T. H., and E. E. Bailey. 1944. Experimental modification and control of molts and changes of coat-color in weasels by controlled lighting. Ann. N. Y. Acad. Sci., **45**: 221–260.

Butler, L. 1947. The genetics of the colour phases of the red fox in the Mackenzie River locality. Can. Jour. Res. D, **25**: 190–215.

COLBERT, E. H., AND J. IMBRIE. 1956. Triassic metoposaurid amphibians. Bull. Amer. Mus. Nat. Hist., **110**: 399–452.

COWAN, I. McT. 1938. Geographic distribution of color phases of the red fox and black bear in the Pacific Northwest. Jour. Mamm., **19**: 202–206.

——. 1947. The timber wolf in the Rocky Mountain National Parks of Canada. Can. Jour. Res. D, **25**: 139–174.

DEFRISE-GUSSENHOVEN, E. 1955. Ellipses équiprobables et taux d'éloignement en biométrie. Bull. Inst. Royal Sci. Nat. Belgique, **XXXI** (26). Bruxelles.

GRÜNEBERG, HANS. 1952. The genetics of the mouse. (2nd ed.) xiv + 650 pp. Martinus Nijhoff, The Hague.

HILDEBRAND, M. 1952. An analysis of body proportions in the Canidae. Amer. Jour. Anat., **90**: 217–256.

HOTELLING, H. 1954. Multivariate analysis. In Kempthorne and others, 1954: 67–80.

HUXLEY, J. S. 1938. Clines: an auxiliary taxonomic principle. Nature, **142**: 219.

HUXLEY, T. H. 1880. Cranial and dental characters of the Canidae. Proc. Zool. Soc. London, 1880: 238–287.

KELSALL, J. P. 1957. Continued barren-ground caribou studies. Wildl. Man. Bull. 1, 12. Canadian Wildlife Service, Ottawa.

KEMPTHORNE, O., AND OTHERS (editors). 1954. Statistics and mathematics in biology. vii + 632 pp. Iowa State College Press, Ames.

KENDALL, M. G. 1957. A course in multivariate analysis. 185 pp. Charles Griffin & Co. Ltd., London.

KOTAKA, TAMIO. 1953. Variation of Japanese *Anadara granosa*. Trans. Proc. Paleont. Soc. Japan, N.S., No. 10: 31–36.

LI, C. C. 1955. Population genetics. xi + 366 pp. The University of Chicago Press, Chicago.

MILLER, G. S., AND R. KELLOG. 1955. List of North American Recent mammals. U. S. Nat. Mus. Bull. 205: 954. Smithsonian Institution, Washington.

MURDOCH, D. C. 1957. Linear algebra for undergraduates. xi + 239 pp. John Wiley and Sons, Inc., New York.

OLSON, E. C., AND R. L. MILLER. 1958. Morphological integration. xv + 317 pp. The University of Chicago Press, Chicago.

QUENOUILLE, M. H. 1952. Associated measurements. x + 242 pp. Butterworths Scientific Publications, London.

RAND, A. L. 1954. The ice age and mammal speciation in North America. Arctic, **7**(1): 31–35.

RAO, C. R. 1952. Advanced statistical methods in biometric research. xvii + 390 pp. John Wiley and Sons, Inc., New York.

RASQUIN, P., AND L. ROSENBLOOM. 1954. Endocrine imbalance and tissue hyperplasia in teleosts maintained in darkness. Bull. Amer. Mus. Nat. Hist., **104**: 359–426.

RAUSCH, R. 1951. Notes on the Nunamiut Eskimo and mammals of the Anaktuvuk Pass Region, Brooks Range, Alaska. Arctic, **4** (3): 147–195.

SCOTT, J. H. 1957. Muscle growth and function in relation to skeletal morphology. Amer. Jour. Phys. Anthrop., N.S., **15** (2): 197–234.

STOCKARD, C. R., AND OTHERS. 1941. The genetic and endocrinic basis for differences in form and behavior as elucidated by studies of contrasted pure-line dog breeds and in biometric research. xvii + 390 pp. John their hybrids. xx + 775 pp. Amer. Anat. Mem. 19. Wistar Inst. Anat. Biol., Philadelphia.

TEISSIER, G . 1955. Allométrie de taille et variabilité chez *Maia squinado*. Arch. Zool. Expér. et Gén., **92**: 221–264.

TODD, T. W., AND R. E. WHARTON. 1934. The effect of thyroid deficiency upon skull growth and form in the sheep. Amer. Jour. Anat., **55**: 97–115.

WRIGHT, S. 1932. General, group and special size factors. Genetics, **17**: 603–619.

YATES, F. 1950. The place of statistics in the study of growth and form. Proc. Royal Soc. B, **137**: 479–489.

YOUNG, S. P., AND E. A. GOLDMAN. 1944. The wolves of North America. xx + 636 pp. American Wildlife Institute, Washington.

BARRIERS TO GENE EXCHANGE WITHIN *MIMULUS GUTTATUS* (SCROPHULARIACEAE) [1]

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In the spring of 1948, Drs. Jens Clausen, David D. Keck, and William M. Hiesey suggested to the author that *Mimulus guttatus* Fisch. and its related species would be a promising group of plants for an experimental study of the processes of evolution. Following their suggestion, such a study was undertaken (Vickery, 1951, 1952). The first part of the investigation, which is the subject of this report, consists of a series of experiments designed to determine the presence and nature of barriers to gene exchange within *M. guttatus,* the main species of the group.

Mimulus guttatus comprises many polymorphic populations growing in moist places such as stream banks, meadows, and springs from the Aleutian Islands to Mexico and from the California coast to the Rocky Mountains. Members of the species vary markedly in height, leaf shape and pubescence, anthocyanin pigmentation of the stem and leaves, flower size and markings, and even in being annual or perennial. However, they are always herbaceous and generally are freely rooting at the nodes. They all have sessile floral leaves, bilabiate, yellow corollas, sensitive stigmas, and inflated mature calyxes. For more detailed descriptions, see Grant (1924) and Pennell (1951).

For this investigation, a living collection of cultures of over two hundred different populations of *Mimulus guttatus* was assembled from all parts of the geographic range of the species. The plants were grown in the greenhouses of the Carnegie Institution of Washington, Stanford, California and of the University of Utah, Salt Lake City, Utah. Fifteen cultures were selected from the living collection for a thorough and intensive study of their crossing behavior. The cultures were chosen with great care to represent the morphological and ecological diversity of the species (see table 1). The selected cultures come from an approximate transect of the range of the species from Pacific Grove on the California coast to Bear Lake in Idaho.

METHODS

In order to detect the presence of sterility barriers initially present within cultures of the selected populations, data were gathered on the number of seeds set by the flowers of each culture in relation to the number of ovules and pollen grains present (see tables 2 and 3). The number of ovules was determined from actual counts of an average of 15 flowers taken from five different plants of each culture. The number of pollen grains was based on counts of an average of four undehisced anthers from different flowers of each culture.

[1] Approximately one half of this work was done at the Carnegie Institution of Washington's Department of Plant Biology, Stanford, California, and was submitted to the faculty of Stanford University as part of the author's Ph.D. dissertation. The other half of the work was carried out at the University of Utah where it was supported by grants from the University of Utah Research Fund and the National Science Foundation.

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TABLE 1. *Origin and characteristics of races of* Mimulus guttatus *used for intraspecific hybridizations*

Culture Number	5001	4929	5004
Origin and collector	Shaded seep, 2 m. elev., Pacific Grove, Monterey Co., Calif., Vickery 1	Sunny, moist swale, 7 m. elev., Pescadero, San Mateo Co., Calif., Clausen 2083	By a spring in the chaparral, 1,500 m. elev., Monterey Co., Calif., Vickery 3
Reference, Univ. of Utah Herbarium no.	28,850	28,849	28,852
Plant height and type *	Tall, facultative perennial 30–110 cm.	Tall, facultative perennial 30–100 cm.	Tall, facultative perennial 35–105 cm.
Leaf characteristics	Ovate, glabrous, dotted with anthocyanin	Ovate with revolute margins, pubescent to sparsely pubescent, suffused with anthocyanin	Ovate, pubescent occasionally patterned with anthocyanin
Flower size, length × width	3.2 × 3.2 cm.	4.0 × 3.2 cm.	2.9 × 1.9 cm.
Culture Number	**5052**	**5346**	**5006**
Origin and collector	Sunny, moist roadside ditch, 370 m. elev., Mt. Diablo, Contra Costa Co., Calif., Stebbins 703	Sunny, gravelly stream bank, 330 m. elev., Mt. Oso, Stanislaus Co., Calif., Vickery 190	Sunny, ephemeral, rocky creek, 430 m. elev., Yosemite Junction, Tuolumne Co., Calif., Hiesey 560
Reference, Univ. of Utah Herbarium no.	28,877	28,922	28,854
Plant height and type *	Tall, facultative perennial 25–90 cm.	Tall, facultative perennial 30–80 cm.	Short, obligate annual 3–30 cm.
Leaf characteristics	Ovate, pubescent to glabrous, occasionally patterned with anthocyanin	Lanceolate, basally laciniate, glabrous; partially patterned with anthocyanin	Broadly ovate, glabrous, anthocyanins show only after frost
Flower size, length × width	3.5 × 2.2 cm.	2.7 × 1.9 cm.	3.0 × 2.7 cm.
Culture Number	**5007**	**5009**	**5010**
Origin and collector	Sunny, marshy meadow, 450 m. elev., Yosemite Junction, Tuolumne Co., Calif., Hiesey 559	Sunny, boggy meadow 1,530 m. elev., Mather, Tuolumne Co., Calif., Hiesey 571	Shady, moist, swale, 1,600 m. elev., Mather, Tuolumne Co., Calif., Hiesey 569
Reference, Univ. of Utah Herbarium no.	28,855	28,856	28,851

* Figures for height are only approximate since environment affects plant size enormously. Plants are perennial only under favorable environmental conditions.

TABLE 1. *Origin and characteristics of races of* Mimulus guttatus *used for intraspecific hybridizations—(Continued)*

Culture Number	5007	5009	5010
Plant height and type *	Tall, facultative perennial 20–80 cm.	Medium, facultative perennial 15–40 cm.	Medium, facultative perennial 20–30 cm.
Leaf characteristics	Lyrate, glabrous, partially patterned with anthocyanins	Ovate, sparsely pubescent, occasionally patterned with anthocyanins	Ovate, pubescent to sparsely pubescent, occasionally patterned with anthocyanins
Flower size, length × width	3.5 × 2.7 cm.	2.2 × 1.9 cm.	2.1 × 1.6 cm.
Culture Number	**5014**	**5015**	**5834**
Origin and collector	Sunny, cascading stream, 2,670 m. elev., Lee Vining Canyon, Mono Co., Calif., Clausen 2039	Shady, rushing stream, 2,150 m. elev., Mono Inn, Mono Co., Calif., Clausen 2043	Sunny stream bank in a marsh, 1,470 m. elev., Salt Lake City, Salt Lake Co., Utah, Vickery 330
Reference, Univ. of Utah Herbarium no.	28,860	28,861	47,247
Plant height and type *	Medium, facultative perennial 20–40 cm.	Tall, facultative perennial 40–150 cm.	Medium, normally perennial 15–35 cm.
Leaf characteristics	Ovate, pubescent to glabrous, no anthocyanins present	Ovate, pubescent to glabrous, no anthocyanins present	Ovate, glabrous, no anthocyanins present
Flower size, length × width	3.0 × 2.7 cm.	3.0 × 2.9 cm.	2.4 × 2.2 cm.
Culture Number	**5835**	**5837**	**5839**
Origin and collector	Shady, moist roadside ditch, 1,600 m. elev., Centerville, Davis Co., Utah, Vickery 331	Sunny seep at edge of Bear Lake, 2,030 m. elev., Bear Lake Co., Idaho, Vickery 322	Shady bank of small stream, 2,350 m. elev., Spruces, Big Cottonwood Canyon, Salt Lake Co., Utah, Vickery 334
Reference, Univ. of Utah Herbarium no.	(unmounted)	28,930	(unmounted)
Plant height and type *	Medium, facultative perennial 20–45 cm.	Medium, facultative perennial 20–40 cm.	Short, perennial by rhizomes 10–44 cm.
Leaf characteristics	Ovate, glabrous, no anthocyanins present	Lanceolate, glabrous, no anthocyanins present	Ovate, glabrous, no anthocyanins present
Flower size, length × width	2.0 × 1.4 cm.	1.9 × 1.3 cm.	2.5 × 1.9 cm.

* Figures for height are only approximate since environment affects plant size enormously. Plants are perennial only under favorable environmental conditions.

TABLE 2. *Relationship of seed set in the parental cultures to the number of ovules and the amount of pollen*

Race	Average no. of self-pollinated seed set per capsule	Average no. of ovules per ovary	Average % of ovules developing into seeds	Average amt. of pollen per anther*	Average % of pollen that functioned	Average % of shriveled pollen
5001	173	1,065	16.2	2,594	6.67	4
4929	127	820	15.5	1,996	6.36	3
5004	114	1,084	10.5	2,402	4.75	2
5052	59	960	6.1	1,474	4.00	30
5346	63	407	15.5	1,326	4.75	14
5006	25	297	8.4	730	3.42	1
5007	43	536	8.0	1,684	2.55	2
5009	11	290	3.8	1,830	0.60	2
5010	27	296	9.1	622	4.34	7
5014	46	504	9.1	1,510	3.05	4
5015	70	1,206	5.8	3,370	2.08	5
5834	5	527	0.9	454	1.10	81
5835	14	590	2.4	452	3.10	94
5837	61	708	8.6	1,800	3.89	4
5839	125	1,086	11.5	2,660	4.70	11
Average	64	692	8.7	1,660	3.69	18

* The approximate amount of pollen generally applied to the stigma in each pollination.

In order to obtain as complete a picture as possible of the intraspecific barriers to hybridization in *M. guttatus,* all the possible interpopulation combinations were attempted (see table 4). For each combination, four flowers, on the average, were hand pollinated. The flowers were emasculated to prevent contamination by their own pollen. In the first experiments, foreign pollen was excluded by covering each flower with a manilla paper envelope or by placing the plant in an insect proof cage. In the later experiments, such contamination was avoided by simply removing the corollas, thereby leaving the pistils bare and unattractive to insects. Every effort was made to harvest the seeds promptly as they are shed soon after ripening. The number of seeds set for each combination was carefully estimated and many of the estimates were checked by actual counts (Vickery, 1956). The success of each hybridization was gauged by sowing the seeds produced and examining the resulting plants.

The fertility of the F_1 hybrids resulting from the initial crosses was determined by hand pollinating an average of 11 flowers on one, two, or occasionally more plants of each combination. In many cases, open pollinated flowers were harvested in addition to the hand pollinated ones (Vickery, 1956). The seed set of the open pollinated flowers averaged only 54% of that of the hand pollinated flowers due probably to a scarcity of insect pollinators in the greenhouses. As would be expected, the open pollinated seeds produced a few seedlings of doubtful parentage. Nevertheless, the open pollinated

TABLE 3. *Correlations between parental characteristics that are potentially related to seed set*

Characteristics of parental cultures	Correlation (r)	Confidence level (p)
Seed set vs. ovule number	0.745	0.01
Seed set vs. amount of pollen	0.715	0.01
Ovule number vs. amount of pollen	0.783	0.01
Flower size vs. seed set	0.502	0.06
Flower size vs. ovule number	0.364	0.15
Flower size vs. amount of pollen	0.450	0.15
Flower size vs. plant height	0.573	0.03
Plant height vs. seed set	0.580	0.03
Plant height vs. ovule number	0.718	0.01
Plant height vs. amount of pollen	0.743	0.01

TABLE 4. *Summary of the seed sets of the interpopulation combinations and their F_1 hybrids*

Cult.		Cultures used as male parents: 5001	4929	5004	5052	5346	5006	5007	5009	5010	5014	5015	5834	5835	5837	5839	Total	Cult.
5001	initial cross	173	150	143	116	118	99	108	92	100	109	122	10	14	117	149	1,298	5001
	F_1 hybrid		92	18	9	81	-	-	-	-	-	13	-	-	14	2		
4929	initial cross	240	127	120	93	95	76	85	69	77	86	98	10	14	94	126	1,193	4929
	F_1 hybrid	26		74	107	169	8	113	116	-	184	35	-	-	0	0		
5004	initial cross	115	140	114	129	20	125	59	55	94	130	150	0	3	20	75	1,115	5004
	F_1 hybrid	-	-		38	-	-	-	4		-		0	136	40	25		
5052	initial cross	21	163	85	59	90	50	57	78	70	85	90	2	10	23	20	844	5052
	F_1 hybrid	-	-	-		-	-	-	-	-	61	-	-	83	20	-		
5346	initial cross	58	93	73	42	63	20	57	42	47	13	18	6	0	24	8	501	5346
	F_1 hybrid	-	-	31	104		40	-	-	2	-	72	18	0	139	31		
5006	initial cross	28	36	15	27	12	25	43	50	35	35	13	10	9	13	8	334	5006
	F_1 cross	13	22	4	2	-		18	18	2	2	-	12	3	9	0		
5007	initial cross	47	76	45	50	9	71	43	44	20	19	38	0	0	13	25	457	5007
	F_1 hybrid	6	143	27	64	24	6		7	11	-	-	0	0	5	7		
5009	initial cross	33	50	20	15	3	44	64	11	24	45	12		6	8	2	326	5009
	F_1 hybrid	15	-	-	46	29	-	-		2	-	9		20	6	0		
5010	initial cross	25	12	27	29	12	9	87	8	27	7	11	10	7	20	5	269	5010
	F_1 hybrid	2	-	4	-	-	-	-	-		-	-	0	0	6	0		
5014	initial cross	76	54	50	70	26	18	88	60	80	46	61	2	25	7	30	647	5014
	F_1 hybrid	10	-	24	-	4	-	26	2	2		6	25	29	125	0		
5015	initial cross	129	133	279	167	90	21	81	50	130	125	70	25	36	100	100	1,460	5015
	F_1 hybrid	-	-	10	50	-	11	28	-	-	4		54	37	33	37		
5834	initial cross	8	13	13	13	7	10	7	3	10	8	10	5	0	8	20	130	5834
	F_1 hybrid	0	7	2	9	-	-	0	0	-	8			0	-	-		
5835	initial cross	15	7	10	13	15	10	10	7	4	25	13	0	14	1	13	143	5835
	F_1 hybrid	86	44	-	-	16	-	4	-	-	-	-	0		-	-		
5837	initial cross	33	38	33	0	25	50	38	25	25	30	50	67	5	61	12	431	5837
	F_1 hybrid	-	41	-	-	-	-	-	-	-	-	-	7	-		-		
5839	initial cross	40	30	250	100	120	15	0	0	50	60	50	15	5	2	125	737	5839
	F_1 hybrid	-	-	-	36	-	-	0	0	-	-	-	24	60	0			
	Total	868	1,017	1,077	964	503	515	807	473	723	674	669	139	106	259	331	9,125	
Cult.		5001	4929	5004	5052	5346	5006	5007	5009	5010	5014	5015	5834	5835	5837	5839		Cult.

For full data see Vickery, 1956. Note that the dash (-) indicates a combination that was made but not tested. In all such cases the reciprocal combination was tested.

TABLE 5. *Comparison of seed set in an F_2 population, F_1 hybrid and their parental cultures*

	Culture number and origin		Av. seed set
P_1 ♀	5346, Mt. Oso, California		63
P_1 ♂	5839, Big Cottonwood Canyon, Utah		125
F_1	1709, 5346 × 5839		31
F_2	5923, 1709 × self	−7	8
		−13	10
		−14	7
		−23	6
		−27	non-flowering
		−28	21
		−34	109
		−49	27
		−54	116
		−70	133
		−71	non-flowering
		−72	5
	Average of live F_2 plants		38*
	plus, 13 plants that died		0
	Average of all F_2 plants		19

* Based on an average of 15 flowers self-pollinated on each plant.

seed set data proved to be a good indication of the fertility of the F_1 hybrids and are included in table 4.

Six selected populations of F_2 hybrids were examined for evidences of hybrid breakdown, that is, loss of vigor or fertility (see table 5). The analysis centered on the factor of vigor which was gauged by measurements of each plant's height and speed of flowering (see table 6).

DISCUSSION OF RESULTS

Seed set varies in the different populations of *M. guttatus* from one or two to over 200 seeds per capsule. It varies from flower to flower, plant to plant, and population to population. However, large, consisent differences in the average seed sets were observed when the fifteen cultures studied were grown in the same environment (see table 2). Apparently, the range of seed set of each population of the species is under genetic control as well as being strongly subject to environmental influences. The genetic control of seed set appears to act by determining the characteristics upon which seed set directly depends, such as the number of ovules present in the flower, the proportion of ovules capable of maturing, the amount of pollen produced, and the proportion of pollen capable of functioning. These characteristics seem to depend, at least in part, on the size of the flower parts and of the plant (see tables 1 and 3). A general size relationship appears to exist in which tall vigorous plants with large flowers produce more pollen and ovules, and hence set more seeds, than do small plants. The relatively continuous variation observed from population to population in all of these characteristics suggests that each trait is controlled

TABLE 6. *Comparisons of flowering and growth in F_2 populations and their F_1 and parental cultures*

Culture	Average number of days to first flower	Average height of plants at time of first flowers	Population size	% of plants flowering
P ♀ 4929	119	31.9	36	100
P ♂ 5006	93	10.7	18	94
F_1 5414	92	38.1	17	100
F_2 5665	104	32.3	154	100
P ♀ 4929	119	31.9	36	100
P ♂ 5009	138	18.2	18	100
F_1 5416	98	42.2	17	100
F_2 5666	125	27.1	483	99
P ♀ 5346	95	29.7	18	100
P ♂ 5006	93	10.7	18	94
F_1 5432	85	25.5	2	100
F_2 5674	106	20.8	769	99
P ♀ 5015	132	26.7	18	94
P ♂ 5007	95	18.8	18	100
F_1 5477	99	50.0	18	100
F_2 5675	111	36.2	502	99
P ♀ 5346	96	21.0	4	100
P ♂ 5839	106	27.5	1	100
F_1 1709	100	32.3	4	100
F_2 5923	101	33.7	49	80

by numerous genes. Thus, the genetic control of the seed set of the different populations of the species depends on the interaction of the several gene systems controlling the development of the ovules and pollen and their contained gametes.

The average seed sets of the fifteen cultures under investigation displayed a spectrum of variation from five to 173 seeds per capsule. The lower end of this spectrum consisted of cultures 5006, 5009, 5010, 5834 and 5835, which exhibited various combinations of low pollen production, ovule formation, or low proportions of functional pollen or ovules. Each of these five cultures contains a cytological abnormality which could account for its below average seed set. For example, cultures number 5009 and 5010 from the Mather, California populations, frequently exhibit lagging chromosomes during the first division of meiosis (Vickery and Mukherjee, in press) with the result that they form numerous microspores with 13, 15, or 16 chromosomes each instead of the 14 regularly present in all the other races of the species. Cytological observations of F_1 hybrids involving these cultures showed that, usually, only gametes with $n = 14$ chromosomes functioned. Therefore, it is not surprising to observe low seed sets in these two cultures. In contrast, the very low fertilities of cultures 5834 and 5835 from populations in the Salt Lake Valley are probably due to the strikingly low amounts of viable pollen they produce (see table 2). The cause of the pollen abortion is not clear, but may be connected with the observed presence of one or two translocations in their chromosomes (Mukherjee and Vickery, unpublished). Lastly, the low seed set of culture 5006 from the Yosemite Junction population is probably related to the tetraploid-like behavior of its chromosomes in meiosis. Frequent bivalents were observed in the second division of meiosis, although no irregular segregations of the chromosomes were actually detected (Mukherjee and Vickery, unpublished). These five cultures with the markedly low seed sets contain all the cytological abnormalities which have been observed in the fifteen populations under investigation. (Vickery, 1955; Mukherjee, Wiens and Vickery, 1957; Mukherjee and Vickery, in press and unpublished). Therefore, their low seed sets are probably not merely normal intraspecific variation, but reflect, at least in part, the presence of sterility barriers within these populations. Reduced seed set apparently can be caused by genes, as well as by chromosomal abnormalities, for example, culture 5015, which exhibits regular chromosome behavior, but nevertheless, has a strong pollen sterility factor which reduces its potential seed set by half (see table 2). Including culture 5015 a total of six of the fifteen populations set fewer seeds than expected on the basis of the number of ovules and the amount of pollen they produce due to the presence of moderately large sterility factors within the different populations of *M. guttatus*. The importance of these sterility barriers is difficult to gauge as all of the populations from which the cultures were taken appear to persist readily in nature.

The sterility factors detected within the six *M. guttatus* populations also may be observed in the seed sets of their respective interpopulation combinations (see fig. 1). The two Salt Lake Valley cultures, 5834 and 5835, show similarly low seed sets. Careful analysis of the data (Vickery, 1956) indicates to the author that the sterility factor in culture 5835 behaved as a dominant. It depressed the seed sets of its interpopulation combinations to levels approximately equal to its own seed set (see table 4). The barrier in culture 5834 behaved as a partial dominant although it probably consists of two separate factors, one like the barrier in culture 5835 and the other, a self-incompatibility factor. These two barriers tended to limit the seed sets of combinations of culture 5834 to values about twice that of the self-pollinated seed set of culture 5834. The sterility barriers in cul-

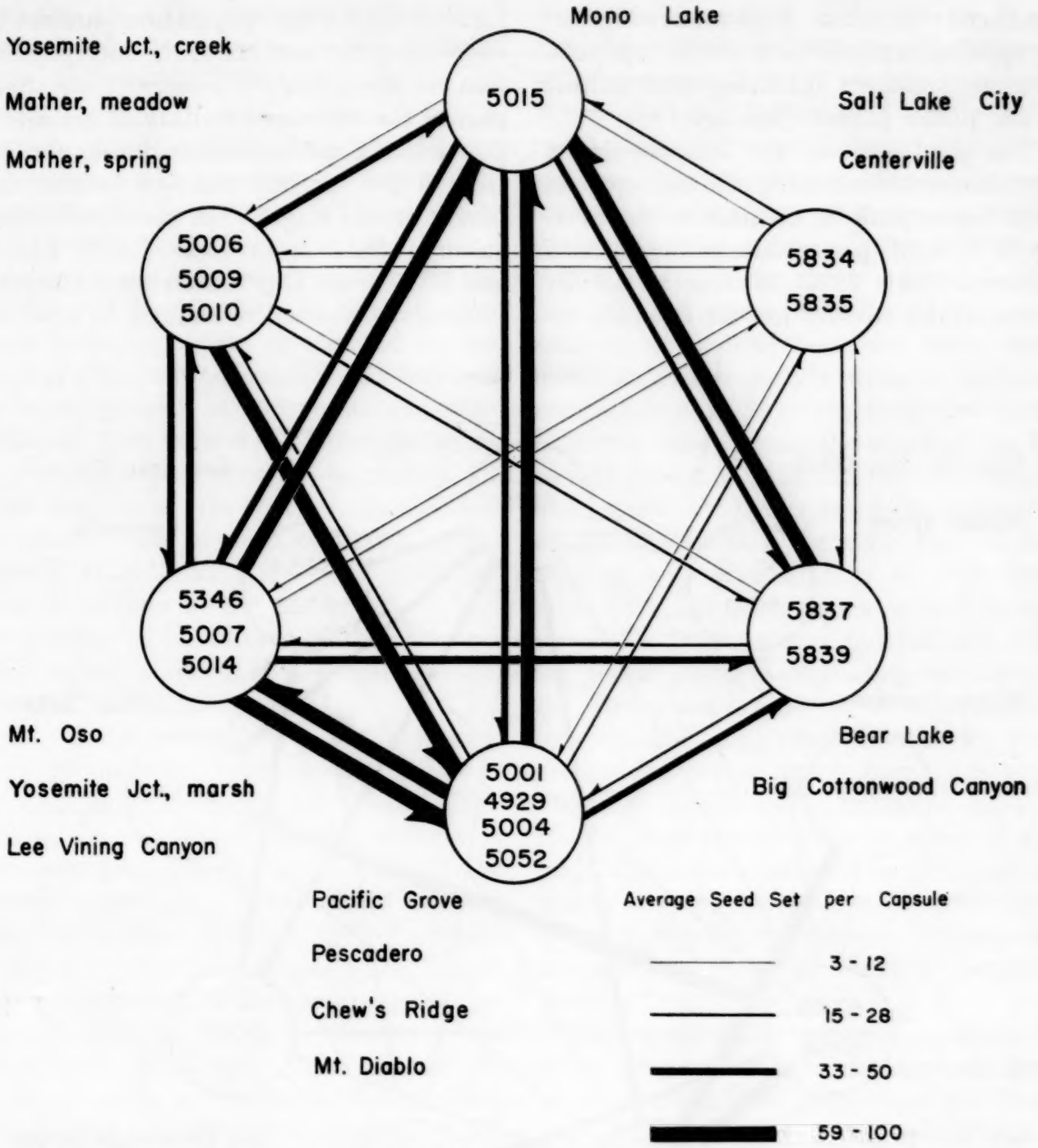

FIG. 1. Summary of seed sets of the interpopulation combinations. The data for cultures that behaved similarly in their interpopulation combinations are lumped for simplicity of presentation (see table 4). The data for reciprocal combinations are indicated by parallel arrows; for example, in the combination 5015 × Salt Lake Valley cultures (5834 and 5835) the arrow points towards 5015 when that culture was used as the female parent and away from it when 5015 was used as the male parent.

tures 5006 and 5010 behaved as recessives when these cultures were used as the male parent, but as dominants when the cultures were used as the female parent in interpopulation combinations. These cultures produced relatively few ovules (see table 2) which is probably the cause of the differential effect of their sterility factors. The barrier in culture 5009 acted like the ones in cultures 5006 and 5010 except that it behaved as a partial dominant when the culture was used as the female parent of a combination. This behavior appears to be due to a self-incompatibility factor, like the one in culture 5834, that reduced its own seed set below the expected level (see table 2). As a result, the seed sets of combinations of culture 5009 tended to be limited to approximately twice the self-pollinated seed set of that culture. Lastly,

the barrier in culture 5015 lowers seed set by approximately 50% in the interpopulation combinations involving that culture as the pollen parent (see fig. 1).

The seed sets of the interpopulation combinations (see table 4) averaged 44 seeds per capsule in contrast to the average of 68 seeds per capsule of the parental cultures. This 35% decrease in fertility demonstrates vividly the far-reaching effects of the six interpopulation barriers in decreasing the seed set of the interpopulation combinations. However, a small part of the decreased fertility of the interpopulation combinations is due to the effects of two barriers that first became apparent at this stage. The combinations of cultures 5837 from Bear Lake, Idaho, and 5839 from Big Cottonwood Canyon, Utah show marked reductions in seed set

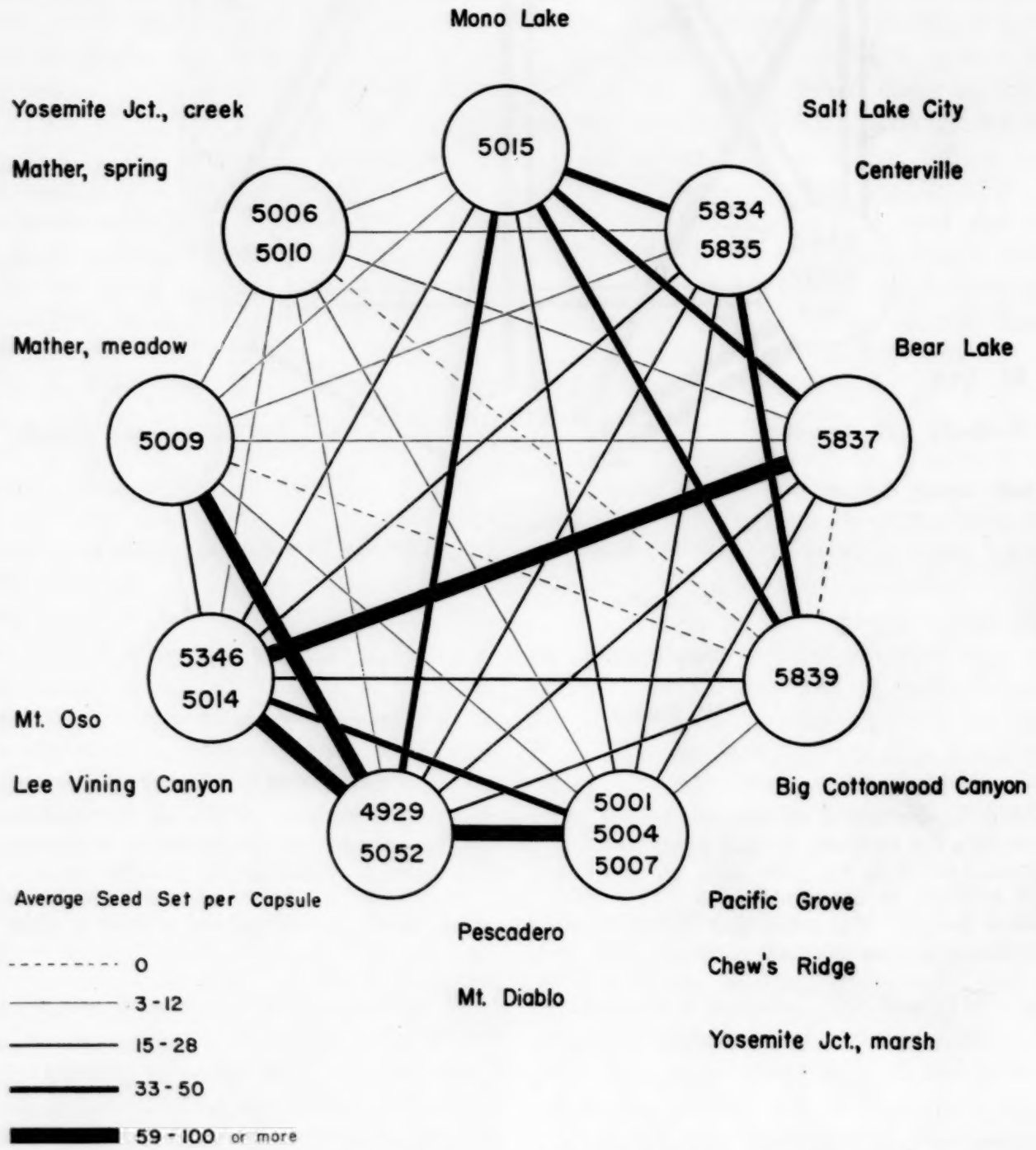

FIG. 2. Summary of seed sets of the interpopulation F_1 hybrids. The data for cultures that behaved similarly in their F_1 hybrids are lumped. Reciprocal differences are not indicated, although in a few cases such differences may exist (table 4). Note that in several cases cultures behaved alike in their interpopulation combinations but differently in their F_1 hybrids. The cultures are regrouped so that such differences may be observed; for example, the decreased fertility of the hybrids of cultures 5001 and 5004 compared to those of 4929 and 5052.

(see fig. 1). The seed sets of combinations involving them as pollen parents were appreciably lower than those in which they were seed parents. These barriers are presumably gene controlled in as much as no chromosomal abnormalities were detected in cultures 5837 and 5839 or in their hybrids.

Seven of the cultures showed strong reductions in the fertility of their F_1 hybrids from the values anticipated on the basis of their self-pollinated seed sets and their performance in interpopulation combinations (see table 4). These decreases in fertility do not seem to be merely intensifications of the barriers previously detected. Four of them occurred in cultures 5006, 5010, 5015, and 5839 which exhibited barriers at the earlier stages and three occurred in cultures 5001, 5004, and 5007 which showed no previous indications of crossing barriers (see fig. 2). Some of the reductions in fertility were very pronounced, for example, those of the F_1 hybrids involving culture 5010. Often, the new barriers took the form of preventing the growth or flowering of the hybrid seedlings. These facts suggest that the barriers in the F_1 hybrid generation plants often may be due to incompatible combinations of the parental gene systems controlling growth, flowering, or seed set. Apparently, the populations represented by the fifteen cultures investigated have accumulated enough gene differences so that they will no longer hybridize without some detriment to many of the offspring. The cumulative effect of all these crossing barriers is to reduce the seed set of the interpopulation F_1 hybrids to an average of 29 seeds per capsule, only 45% of the average of the parental values.

A survey of several F_2 hybrid populations was run to detect the presence of hybrid breakdown, that is, the loss of fertility or vigor in the F_2 generation hybrids due, presumably, to imbalance or incompatibility in the gene systems caused by the reassortment and recombination of the parental chromosomes. In the one small F_2 population that was carefully scored for fertility (see table 5), the seed set ranged from 0 to 133 seeds per capsule. When the plants that died before they could be scored were included, the average was 19 seeds per capsule. The range includes the seed set of the more fertile parent, but the average is below that of the F_1 hybrid or either parent. Apparently, the recombinations of the gene systems controlling seed set were more likely to reduce rather than to increase seed set in this fairly representative combination. In contrast, the vigor of the F_2 hybrid plants, as indicated by their height and speed of flowering, was generally either intermediate, between that of the parents, or exceeded that of both (see table 6). The data on F_2 hybrid breakdown are fragmentary, but indicate that the trend towards decreasing fertility in the successive stages of interpopulation hybridization continues into the F_2 generation, but that complete barriers to gene exchange are unlikely to occur at this stage. Apparently, whereas some of the populations are separated by moderate or strong crossing barriers, in all probability none of the fifteen cultures investigated are separated from the others by complete barriers to gene exchange.

The species *M. guttatus* consists of many geographically isolated populations most of which have evolved partial crossing barriers of various kinds and strengths. In some cases, populations that are closely similar morphologically and ecologically are separated by the strongest barriers, for example, the two populations from the Salt Lake Valley or the two populations from Mather in the Sierra Nevada. The evolution of crossing barriers in *M. guttatus,* like that of those in *Galeopsis* (Muntzing, 1930), appears to be quite independent of the evolution of morphological and physiological characteristics.

Summary

This investigation reveals the presence of a large array of different crossing barriers of various strengths within the

single species, *Mimulus guttatus* Fisch. All but three of the fifteen populations analyzed have barriers of one or more kinds, six of which act within particular populations, two of which become manifest at the stage of initial interpopulation hybridization, and seven of which appear in the interpopulation F_1 hybrids and, as a preliminary analysis indicates, continue into the F_2 hybrid generation. Apparently, the barriers are caused primarily by genes, and secondarily by translocations, meiotic irregularities, and tetraploid-like behavior of the chromosomes. The cumulative effect of all the different barriers is to produce, with a few exceptions, a strong downward trend in intraspecific fertility from that of the self-pollinated parental cultures (64 seeds per capsule) to that of the interpopulation combinations (44 seeds per capsule) to that of the F_1 hybrids (29 seeds per capsule). However, the vigor of the F_2 hybrids indicates that the downward trend probably starts to level off at this stage. Therefore, although most of the populations were found to be separated from each other by moderate to strong crossing barriers, none was completely isolated. Gene exchange was still potentially possible between all the populations investigated. Therefore, the geographically isolated populations, comprising *M. guttatus* are genetically one species, but they have evolved a strikingly large number of barriers to gene exchange. These facts suggests to the author that this widely scattered, highly polymorphic species may well be on its way towards evolving into a host of closely related new species.

Literature Cited

Grant, A. L. 1924. A monograph of the genus *Mimulus*. Annals of the Missouri Botanical Garden, **11**: 99–388.

Pennell, F. W. 1951. *In* Abram's Illustrated flora of the Pacific States. **3**: 688–731. Stanford Univ. Press, Stanford, California.

Muntzing, A. 1930. Outlines to a genetic monograph of *Galeopsis*. Hereditas, **13**: 185–341.

Mukherjee, B. B., D. Wiens, and R. K. Vickery, Jr. 1957. Chromosome counts in the section *Simiolus* of the genus *Mimulus* (Scrophulariaceae). II. Madroño, **14**: 128–131.

——, and R. K. Vickery, Jr. 1959. Chromosome counts in the section *Simiolus* of the genus *Mimulus* (Scrophulariaceae). III. Madroño (in press).

Vickery, R. K., Jr. 1951. Genetic differences between races and species of *Mimulus*. Carnegie Institution of Washington Yearbook, **50**: 118–119.

——. 1952. A study of the genetic relationships in a sample of the *Mimulus guttatus* complex. Ph.D. Dissertation, Stanford Univ. California. 253 pp. typescript.

——. 1955. Choromosome counts in the section *Simiolus* of the genus *Mimulus* (Scrophulariaceae). Madroño, **13**: 107–110.

——. 1956. Data on interracial hybridizations in *Mimulus guttatus* (Scrophulariaceae). Proc. of the Utah Acad. of Arts, Sciences and Letters, **33**: 37–43.

NATURAL TRIPLOIDS IN THE ORCHARD GRASS, *DACTYLIS GLOMERATA* L., POLYPLOID COMPLEX AND THEIR SIGNIFICANCE FOR GENE FLOW FROM DIPLOID TO TETRAPLOID LEVELS

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INTRODUCTION

Diploid and tetraploid forms in sexual polyploid groups have been generally regarded as reproductively completely isolated from one another. Whether this is always the case was already questioned by Zohary (1956) and Stebbins and Zohary (1959). In the following work an attempt is made to examine the genetic relationships between diploid and tetraploid populations of the Orchard Grass, *Dactylis glomerata* L., in places where such populations come in contact in Israel. This paper reports on a study of the occurrence of natural triploids in contact areas and on the types of progeny such triploids produce under natural conditions.

Previous studies (Zohary 1956, Stebbins and Zohary 1959) have established the polytypic group of Orchard Grass (*Dactylis glomerata* L.) as a large polyploid complex. This complex contains at least ten distinct diploid ($2n = 14$) subspecies as well as a large, sexual, tetraploid ($2n = 28$) superstructure. The diploid forms have each a restricted geographical distribution and they are widely separated from one another. Each diploid is characterized also by comparatively narrow and distinct range of variation. In contrast with the diploids, tetraploid *Dactylis* forms are continuously and widely spread over most of Europe, West Asia and North Africa. The tetraploid level is characterized by a large, continuous range of variation which covers most of the characters found in the various diploids. Cytogenetically all tetraploid forms are interfertile and show typical autopolyploid chromosome behavior.

MATERIALS AND METHODS

Seed collected from the triploid plants was planted in the greenhouse at Jerusalem. Seedlings were later transferred to 8″ pots and grown outside.

For cytological study, material was fixed in 3:1 alcohol acetic acid for 24 hours and stored later in 70% alcohol. In plants which flowered, chromosome counts were made in aceto-carmine squashes of microsporocytes. Stages used were anaphase I and diakinesis. At least 8–10 well spread cells were analyzed in each plant. In non-flowering plants chromosome counts were made from shoot meristems. Growing shoots were dissected longitudinally and pretreated for three hours with water solution of paradichlorbenzen and the Feulgen staining and squash method was employed.

To determine the percentage of pollen abortion, pollen grains were stained with 2% aceto-carmine. For each plant examined no fewer than 200 pollen grains were counted.

Seed-set was determined by examination, in each plant, of 50 almost mature spikelets, taken at random from the second and third lower branches of the panicle. Only the lower two florets in each spikelet were examined. A floret was considered fertile when a well developed caryopsis was found in it.

OBSERVATIONS

(1) *Occurrence of Triploids in Contact Areas.* Both diploid ($2n = 14$) and

tetraploid (2n = 28) forms of *Dactylis* are found in Israel (Zohary 1956, Stebbins and Zohary 1959). In the hilly area around the town of Safad, Upper Galilee, these two chromosomal forms are widely and continuously spread. On soft Senonian chalks here, diploid populations have been found to occupy north-facing slopes, while on ridges and on south-facing slopes they are replaced by tetraploid plants (Nur and Zohary 1959). Close contacts between populations are frequent.

A search for natural triploids was conducted in such a place of contact about 200 meters north of Safad. In this location, the contact between the diploid and tetraploid populations was found on a west-facing slope and it consisted of a narrow belt, where diploid and tetraploid plants were found growing together in a mixed population.

This area, about 100 m wide and 150 m long was screened for triploids in the spring of 1957. Triploids in *Dactylis* have already been shown to be highly male-sterile, resulting in non-dehiscence of their anthers (Müntzing 1937). Plants in this area were therefore examined for this morphological trait. Out of about 2000 plants checked, 27 were found to have non-dehiscent anthers.

The next step was cytological examination (meiosis in P.M.C.'s) of the non-dehiscent plants. This was possible in 15 plants. 8 of these were found to be diploids, 4 tetraploids and 3 triploids (No. 1 to No. 3).

A similar check was done in another contact about 300 m east of the previous location. 9 non-dehiscent plants were found, 4 of them triploids (No. 4 to No. 7).

All triploids were quite vigorous and normal in appearance and could not be morphologically distinguished from the adjacent diploids and tetraploids. Careful cytological examination was carried out in the first three triploids. Each was found to have exactly 21 normal chromosomes. In their meiotic behavior these three triploids agree closely with the figures described by Müntzing (1937) in *Dactylis aschersoniana* × *D. glomerata* triploids.

(2) *Seed-set in Triploids.* Two of the triploid plants (No. 1 and No. 2) were marked and left undisturbed in the field. The location of triploid No. 2 was more or less in the middle of the contact zone, while triploid No. 1 grew already in a predominately diploid population. These two triploids were visited again at the end of May 1957 and examined for natural seed-set. Seed-set in the two plants was approximately 5 per cent as compared to values of 25–30 per cent obtained in adjacent diploid and tetraploid plants.

(3) *Progeny of the Triploids.* The seed which was collected from the two triploids was planted in Jerusalem in October 1957. Seed from triploid No. 1 gave rise to 14 seedlings and that of plant No. 2 to 19 seedlings. In both collections, germination was very uneven and already in the seedlings a wide variation in morphology and development was observed. About one third of the progeny showed very weak or retarded growth, which was accompanied in several cases by morphological abnormalities such as very slender shoots, tiny leaves, curling of leaves and tillers. Some of these abnormal seedlings died in early stages of development.

By April 1958 only 23 of the progeny survived. The larger proportion of these were vegetatively normal and well developed plants who came to flower in May and early June. Several other plants looked vigorous vegetatively but produced few or no flowering stalks. Some of the abnormal seedlings with the small, tender leaves and tillers still survived and grew slowly but again did not come to flower. Summary of the observations on the progeny of the triploids is given in tables 1 and 2.

(4) *Cytology of the Progeny of the Triploids.* Chromosome counts made it

TABLE 1. *Progeny from Triploid No. 1*

Plant number	Chromosome number	Morphological characterization	Per cent pollen abortion	Per cent seed-set
DC891–1	28 + 2B	vigorous, many inflorescences	36	32
DC891–2	28	vigorous, many inflorescences	39	44
DC891–3	28 + B	vigorous, few inflorescences	28	30
DC891–4	28	vigorous, few inflorescences	37	32
DC891–5	—	very weak, did not flower	—	—
DC891–6	35 (circa)	weak, only one inflorescence	—	—
DC891–7	28	vigorous, few inflorescences	21	20
DC891–8	28	vigorous with several delicate inflorescences	32	15

possible to classify the progeny of the triploids into the following groups:

(a) Tetraploids. Twelve plants were found to be tetraploids (see tables 1 and 2). Of these, nine plants showed strict tetraploid (2n = 28) chromosome number. The other three had, in addition to the normal tetraploid chromosome set, an extra one or two supernumerary chromosomes (fig. 1) and one (DC 892–15), an extra normal chromosome as well.

Meiosis figures in the tetraploid progeny were very similar to what is observed in regular tetraploid *Dactylis* material (Müntzing 1937, Zohary 1956). Supernumerary chromosomes, when present, resemble closely, in their form (size and staining) and their behavior (precocious movement at M_I, lagging and precocious

TABLE 2. *Progeny from Triploid No. 2*

Plant number	Chromosome number	Morphological characterization	Per cent pollen abortion	Per cent seed-set
DC892–1	28	vigorous, many inflorescences	24	18
DC892–2	28	vigorous, many inflorescences	19	24
DC892–3	35	quite weak vegetatively, broad leaves, only one inflorescence	—	—
DC892–4	35 (circa)	fairly vigorous, few inflorescences	27	26
DC892–5	28	vigorous, fairly many inflorescences	25	4
DC892–6	16	vigorous vegetatively, did not flower	—	—
DC892–7	35	vigorous vegetatively, only one inflorescence	18	—
DC892–8	18	fairly vigorous vegetatively, only one inflorescence	—	—
DC892–9	15	fairly vigorous, only tillers, did not flower	—	—
DC892–10	28	fairly vigorous, few inflorescences	40	17
DC892–11	28	fairly vigorous, many inflorescences	28	28
DC892–12	35	fairly vigorous vegetatively, only one inflorescence	37	—
DC892–13	35	vigorous, leaves and tillers coarse, few inflorescences	15	7
DC892–14	16	weak, did not flower	—	—
DC892–15	29 + B	vigorous, many inflorescences	45	31

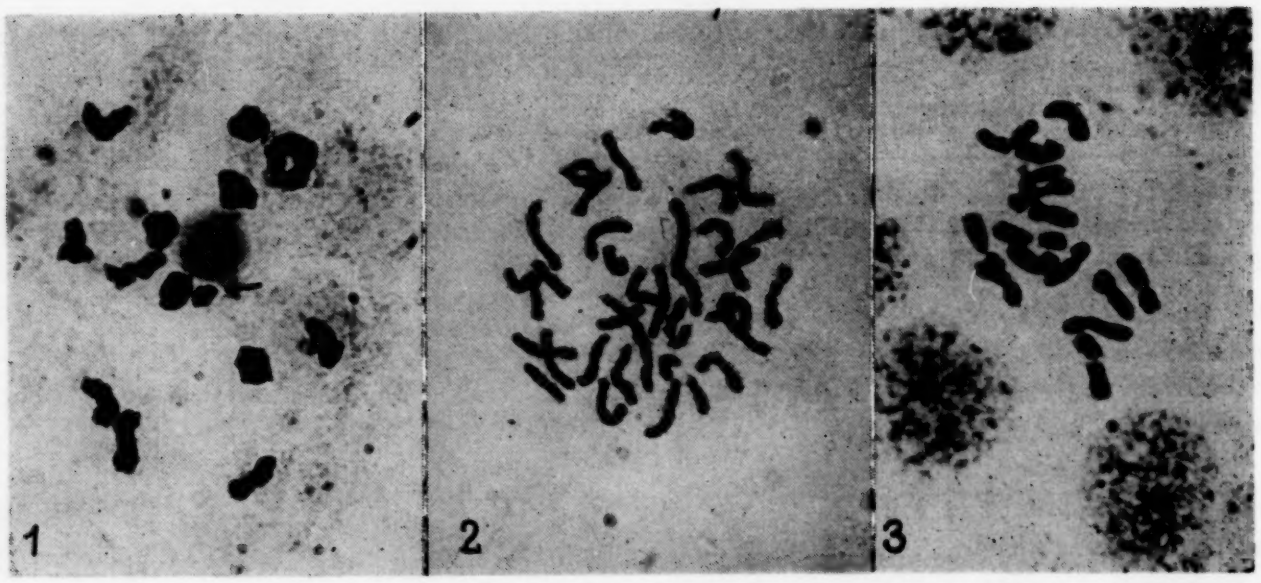

FIG. 1. Microsporocyte in diakinesis in plant DC 891–3 (2n = 28 + B), showing 1 quadrivalent, 12 bivalents and (arrow) a single supernumerary chromosome (× circa 750).
FIG. 2. Metaphase in dividing shoot meristem cell in plant DC 892–7 with 2n = 35 (× circa 750).
FIG. 3. Metaphase in dividing shoot meristem cell in plant DC 892–9 showing 15 chromosomes (× circa 1100).

division at A_I), the B chromosomes which are common in the adjacent diploid population (Zohary and Ashkenazi 1958). The existence of these supernumerary chromosomes was verified also in mitosis of shoot meristems.

(b) Pentaploids. Six plants were classified as pentaploids (see tables 1 and 2). In three of these plants (DC 892–3, DC 892–12 and DC 892–13) good anaphase I figures have been obtained and the 35 elements could be clearly and definitely counted. In another plant (DC 892–4), where meiosis figures were sticky, the chromosome number was estimated to be around 35 (not less than 33). However, it was impossible to verify definitely whether this plant had exactly the pentaploid chromosome number. In plants DC 891–6 and DC 892–7, which almost did not flower, chromosomes were counted in mitosis of shoot meristems (fig. 2).

Morphologically all the six pentaploids were characterized by somewhat coarser leaves and tillers. Only two of them were really vigorous. The development and flowering of the four other plants was much weaker.

(c) Aneuploids. Four aneuploid plants were found among the progeny of the triploids (see table 2), with chromosome numbers of 15, 16 and 18 (fig. 3).

Morphologically all four plants belong to the group of the less vigorous progeny. No one of them flowered.

(d) Plants with Undetermined Chromosome Number. In a single plant, examination of chromosome number was unsuccessful. It was a weak, non-flowering plant (see table 1), in which dividing cells in shoot meristems were very scarce. It is suspected that this plant too, has an aneuploid chromosome number.

DISCUSSION

The most likely explanation for the presence of tetraploid and pentaploid plants among the progeny of the triploids is the assumption that triploids produce an appreciable number of unreduced eggs. When such 3x eggs are fertilized by pollen from adjacent diploid plants, they give rise to tetraploids; when fertilized by pollen from tetraploid plants, they produce pentaploids (see fig. 4). Already Müntzing (1937) assumed production of unreduced eggs to explain occurrence of some pentaploid progeny when crossing triploid *D. glomerata* × *aschersoniana* back to tetraploid *D. glomerata*.

The assumed production of unreduced eggs is strongly supported by another line of evidence—the presence of supernumerary chromosomes in some of the tetraploid progeny. Supernumerary chromosomes have been found to be quite common in diploid *Dactylis* populations in Israel. The diploid population, adjacent to the triploids studied, was extensively investigated (Zohary and Ashkenazi 1958) and found to have a frequency of 0.73 supernumerary chromosomes per plant. In contrast with the diploid material, our cytological survey of *Dactylis* in Israel (Nur and Zohary 1959) revealed no such accessory chromosomes in tetraploid populations. The only exception encountered was a single tetraploid plant near the contact zone itself. Therefore, in the present case, supernumerary chromosomes can be regarded as a cytological marker of the diploid material. They were not found in the two parent triploids. They could only have reached their tetraploid progeny in *haploid* pollen of diploid plants.

Since a large proportion of their viable progeny are tetraploids, triploid *Dactylis* in the contact areas can be regarded as an efficient one-way bridge for gene flow from diploids to tetraploids. If the assumption of unreduced eggs is correct, the resulting tetraploids receive two chromosome sets from the donor diploid population (see scheme in fig. 4, a). The normal development and fertility observed in the polyploid progeny (see tables 1 and 2) is another point that should be stressed. Thus the newly created tetraploids should survive and compete well with ordinary tetraploids.

The present data indicate, therefore, that the reproduction barriers between diploid and tetraploid populations in *Dactylis* are not as complete as was previously assumed in cases of polyploids and that one-way gene flow from diploids to tetraploids does exist. To these indications one has to add still another theoretical possibility of a similar gene flow, in contact areas, brought by occasional formation of unreduced gametes in the diploid plants themselves and their union with 2n gametes produced by tetraploid plants. However, this possibility has yet to be demonstrated. Another point worth mentioning is the full accordance of these data with the varia-

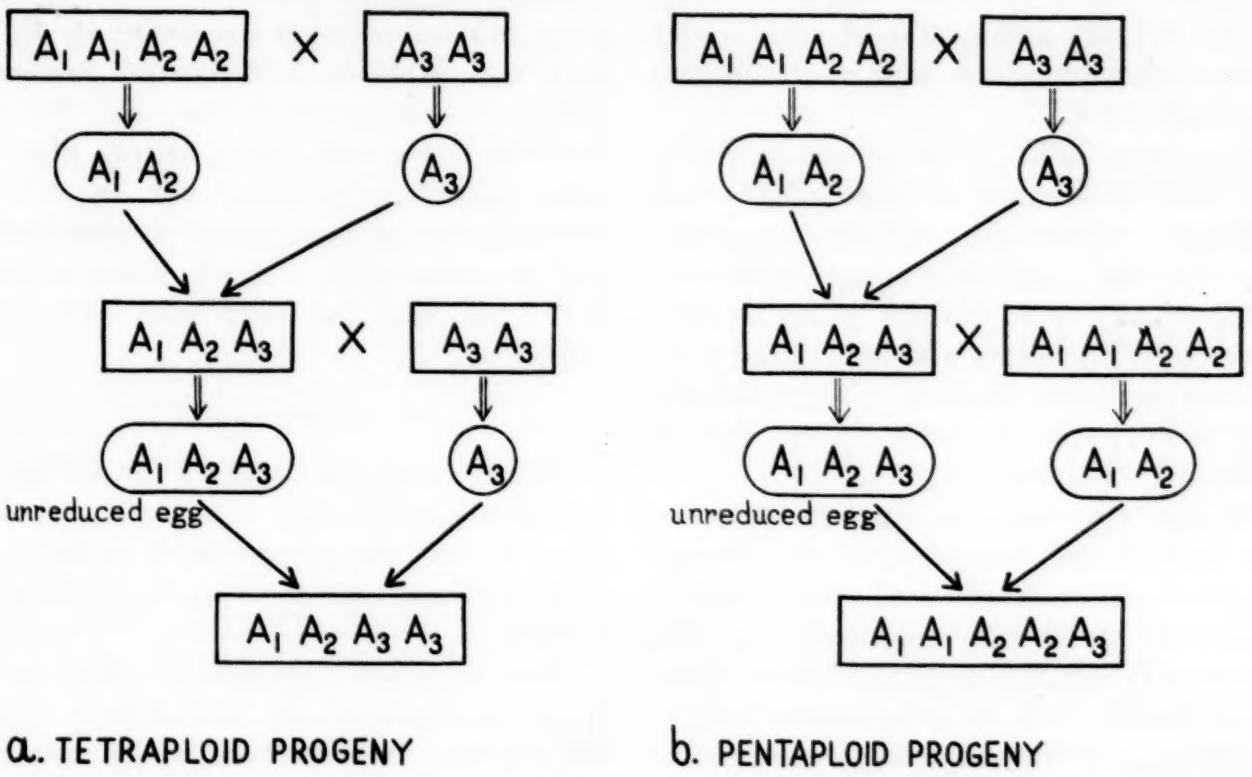

FIG. 4. Schematic representation of the production by triploids of *a*. tetraploid progeny; *b*. pentaploid progeny.

tion patterns found in the diploid and tetraploid populations in areas where they come in contact. In many such cases (Zohary 1956) tetraploid morphology largely overlaps the diploids and separation of the two chromosomal levels from one another on a morphological basis is impossible.

These indications of incomplete genetic barriers between the two chromosomal levels in *Dactylis* are important for general considerations of the mode of evolution of tetraploid superstructures. Two main evolutionary models have been suggested to account for the formation of large, polyploid superstructures such as found in *Dactylis glomerata*.

The first model explains the origin of the superstructure by strict autopolyploidy of its diploid progenitors, followed by fusion on the tetraploid level. This mode of origin seems highly unlikely on the basis of available information (for details see Stebbins 1947, 1950).

The other mode of origin was suggested by Stebbins (1950) who assumed the formation of the tetraploid superstructure as a result of various intervarietal crosses between distinct diploid forms, followed by chromosome doubling in the hybrids and fusion of such initial tetraploid populations into a continuous superstructure.

A serious difficulty in the last model is the need to assume a large number of contacts between the diploids contributing to the polyploid superstructure. Theoretically every diploid "pillar" should have come in contact and hybridized with at least one other diploid. Geographically and historically in some cases this is difficult to visualize.

If the reproductive barriers between the diploid and tetraploid levels are incomplete, the large continuous superstructure needs not necessarily be the result of fusion of a large number of tetraploid forms, each of independent origin. It could have evolved from a small number of initial intervarietal tetraploid combinations which spread to new regions and incorporated genetic material from additional diploid forms via triploids. The work of Heiser (1949, 1951) in *Helianthus annuus* has demonstrated that introgression from related species can greatly enrich the adaptive range of a given species and facilitate its rapid spread. Since the reproductive barrier between diploid and tetraploid levels in *Dactylis* is likewise not absolute, a similar process could have operated here and greatly assisted in the spread of the initial intervarietal polyploids. The advantage of such a model is that one does not need to postulate a large number of direct contacts between the diploid forms themselves to account for the production of all tetraploid combinations necessary to cover the entire range of variation met in the superstructure.

Another point worth mentioning is that introgression via triploids could operate only in cases of autopolyploid chromosome behavior. It could not work in an allopolyploid genetic system. If found of general occurrence in evolutionary successful autopolyploid complexes, it should system.

In conclusion, the present information strongly suggests that the model of the polyploid complex, as presented by Babcock and Stebbins (1938) and Stebbins (1950) is yet a simple one, and that in *Dactylis glomerata,* and possibly also in other sexual polyploid groups, the whole complex should be regarded as genetically and taxonomically a much more closely knit unit than has been previously assumed.

Summary

Natural triploids occur in contact areas between diploid and tetraploid populations of *Dactylis glomerata* in Israel and such triploids set seed in their natural habitat.

The triploids apparently produce a large proportion of unreduced eggs. Fertilization of such eggs by haploid pollen (from diploid plants) and diploid pollen (from tetraploid plants) results

in the formation of tetraploids and pentaploids respectively.

By producing a large proportion of vigorous tetraploid progeny, triploids can serve as an efficient bridge for one-way gene flow from diploid level to tetraploid level. The evolutionary significance of such type of introgression for the construction of tetraploid superstructure in polyploid complexes is pointed out.

Acknowledgments

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Literature Cited

Anderson, E. 1953. Introgressive hybridization. Biol. Rev., **28**: 280–307.

Babcock, E. B. and G. L. Stebbins Jr. 1938. The American species of *Crepis:* their relationships and distribution as affected by polyploidy and apomixis. Carnegie Inst. Washington, Publ. No. 504, 200 pp.

Heiser, C. B. 1949. Study in the evolution of the sunflower species *Helianthus annuus* and *H. Bolanderi.* Univ. Calif. Publ. Bot., **23**: 157–208.

——. 1951. Hybridization in the annual sunflowers: *Helianthus annuus* × *H. debilis* var. *cucumerifolius.* Evolution, **5**: 42–51.

Müntzing, A. 1937. The effects of chromosomal variation in *Dactylis.* Hereditas, **23**: 113–235.

Nur, U. and D. Zohary. 1959. Distribution patterns of diploid and tetraploid forms of *Dactylis glomerata* L. in Israel. Bull. Res. Counc., Israel, Sect. D: Botany, **7D**: 13–22.

Stebbins, G. L. Jr. 1947. Types of polyploids: their classification and significance. Advances in Genetics, **1**: 403–429.

——. 1950. Variation and evolution in plants. New York, Columbia Univ. Press, 641 pp.

—— and D. Zohary. 1959. Cytogenetic and evolutionary studies in the genus *Dactylis.* I. The morphology, distribution and interrelationships of the diploid subspecies. Univ. Calif. Publ. Bot. (in press).

Zohary, D. 1956. Cytogenetic studies in the polyploid complex of *Dactylis glomerata* L. Ph.D. thesis. Univ. of California, Berkeley.

—— and I. Ashkenazi. 1958. Different frequencies of supernumerary chromosomes in diploid populations of *Dactylis glomerata* in Israel. Nature, **182**: 477–478.

POPULATION STUDIES ON *VIOLA LACTEA* SM. AND ITS WILD HYBRIDS

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Introduction

Viola lactea Sm. belongs to the group *Arosulatae* of the sub-section *Rostratae,* section *Nomimium* (Becker, 1910). Its distribution is "Oceanic West European" (Matthews, 1955) and it occurs in scattered localities from the south and west of the British Isles, down the Atlantic coast of France and through N.W. Spain, to reach its southern limit in Portugal about the River Tagus (39°N). It is typically a member of the "atlantic" heath communities of Western Europe and inhabits dry, acid, rather sterile soils. The detailed ecology is described elsewhere (Moore, 1958).

Compared with the eurasiatic distribution of *V. canina* L. (Group Arosulatae), the restricted range of *V. lactea* seems primarily due to climate since its eastern boundary does not extend beyond the 38° F. January isotherm. Where climatic conditions are such that the species are likely to come together, as in S.W. Britain, their differing edaphic requirements have been shown to influence their choice of habitat (Moore, 1958). The most important of these was found to be the base status of the soil; exchangeable calcium is high in soils with *V. canina* (19.17 ± 4.293 m.equiv./100 gms. soil) and low in those with *V. lactea* (5.11 ± 2.719 m.equiv./100 gms. soil). Other factors of importance include competition from associated species, biotic influences and physical stability of the substrate. *V. riviniana* Rchb. (Group *Rosulantes*) is found over the whole of Europe and has been shown (Valentine, 1949, 1950) to have broad climatic and edaphic tolerances.

V. lactea (2n = 58) is an allohexaploid which appears to be derived from the closely related *V. canina* (2n = 40) and a diploid species, as yet unidentified; it has two genomes in common with *canina,* one of which is also present in the tetraploid *V. riviniana* (Moore, unpub.).

V. lactea has been readily crossed with *V. canina* to give a hybrid of reduced fertility and with *V. riviniana* to give one of low fertility. Any consideration of natural hybridization and introgression must take into account the separation in the field of *V. lactea* and *V. canina* by climatic and edaphic factors; on the other hand *V. riviniana* is able to come into wide contact with *V. lactea* over much of its range so that opportunities for crossing are frequent. It may be noted here that *V. riviniana* also overlaps with *V. canina* in the field and their sterile interspecific hybrid is often encountered.

Records from the literature and from herbaria suggested that the wild hybrids *V. canina* × *lactea* and *V. lactea* × *riviniana* occur fairly frequently in Britain and Europe. However, many diagnostic characters of the violets do not normally preserve well on drying and the use of pressed specimens has led to confused determination of these hybrids and their derivatives. This problem seemed most readily approached by analyzing wild populations, including *V. lactea,* using methods such as those described by Anderson (1949).

This account describes attempts to assess the occurrence and extent of hybridization involving *V. lactea,* and to utilize such data in evaluating the pres-

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ent field status and evolutionary position of this species.

Material and Methods

Field methods. During the early summer months of 1955–57, a comprehensive range of violet populations including *V. lactea* was studied in many localities in S.W. Britain and Portugal. Samples of material for use in biometrical analyses were collected.

Certain populations, needed to act as marker samples, or fixed points, in the analyses, were determined as typical of the three species and were taken from characteristic habitats in representative localities. Samples were also taken from vigorous clumps of plants which, on morphological grounds, appeared near the artificial interspecific hybrids; these could not be random samples since each clump probably constituted a single clone. The remaining populations sampled were suspected to be intermediates either because of habitat, locality or morphology.

A random sample of 30 plants was taken from most populations. While still fresh, the petal color was scored; one flower was then removed from each plant and the rest of the plant labelled and pressed. The spur petal was detached and, together with those from the rest of the sample, affixed to a card (Heslop Harrison, 1949) and labelled to correspond with the leaves. The remainder of each flower was preserved in alcohol to retain the style and pollen for later examination.

Characters. The following eight characters were used for distinguishing the species.

1. Ratio of length along midrib/greatest breadth of leaf.

2. Basal angle of leaf (angle between petiole and lower edge of lamina to denote degree of cordate- or cuneate-ness). The leaf taken was that which subtended the uppermost flower.

These characters adequately define leaf shape and are those which are most consistently found on herbarium specimens. Russell (1952, 1954, 1956) used them in studies of acaulescent violets.

3. Ratio of greatest length/greatest breadth of spur petal.

4. Apical angle of spur petal (angle between line of mid-vein and the edge of the petal).

5. Flower color. Scored in the field by comparison with a color chart covering the range of colors involved. The flower colors in *V. canina, V. lactea* and *V. riviniana,* denoted respectively in the chart by index numbers 1, 3 and 5, correspond to the following horticultural color standards of Wilson (1938), the spectrum hue number being given in brackets: 1, Gentian Blue (42); 3, Pale Cobalt Violet ($^{6}34/_{3}$); 5, Violet (36). All the hybrid plants involved in this work were rated on the basis of these indices.

6–7. Length of style and length of longest stigmatic papilla. Measured by means of a micrometer eye-piece to characterize style shape which was used by Clausen (1929) and Gershoy (1934) in determining violet species.

8. Percentage pollen fertility.

Methods of Analysis. Comparison of these violet populations using single characters is unsatisfactory because of morphological overlap, and it is necessary to combine several characters in order to delimit species and hybrids. The ratio of style length/length of the longest stigmatic papilla gives a good separation of the species (Moore, 1957a) but is unsatisfactory for determining many intermediate populations.

Three different methods for combining the characters into a single expression were tried and "Polygonal Graphs" were finally adopted. Since *V. canina* lies between the other species in most characters (table 1) Anderson's "Hybrid Index" is of little use. The "Discriminant Function" (Fisher, 1936) also proved unsatisfactory, though it would be valuable for studying the more subtle intraspecific differences. Polygonal graphs have been employed for simultaneously presenting a number of variables by several workers

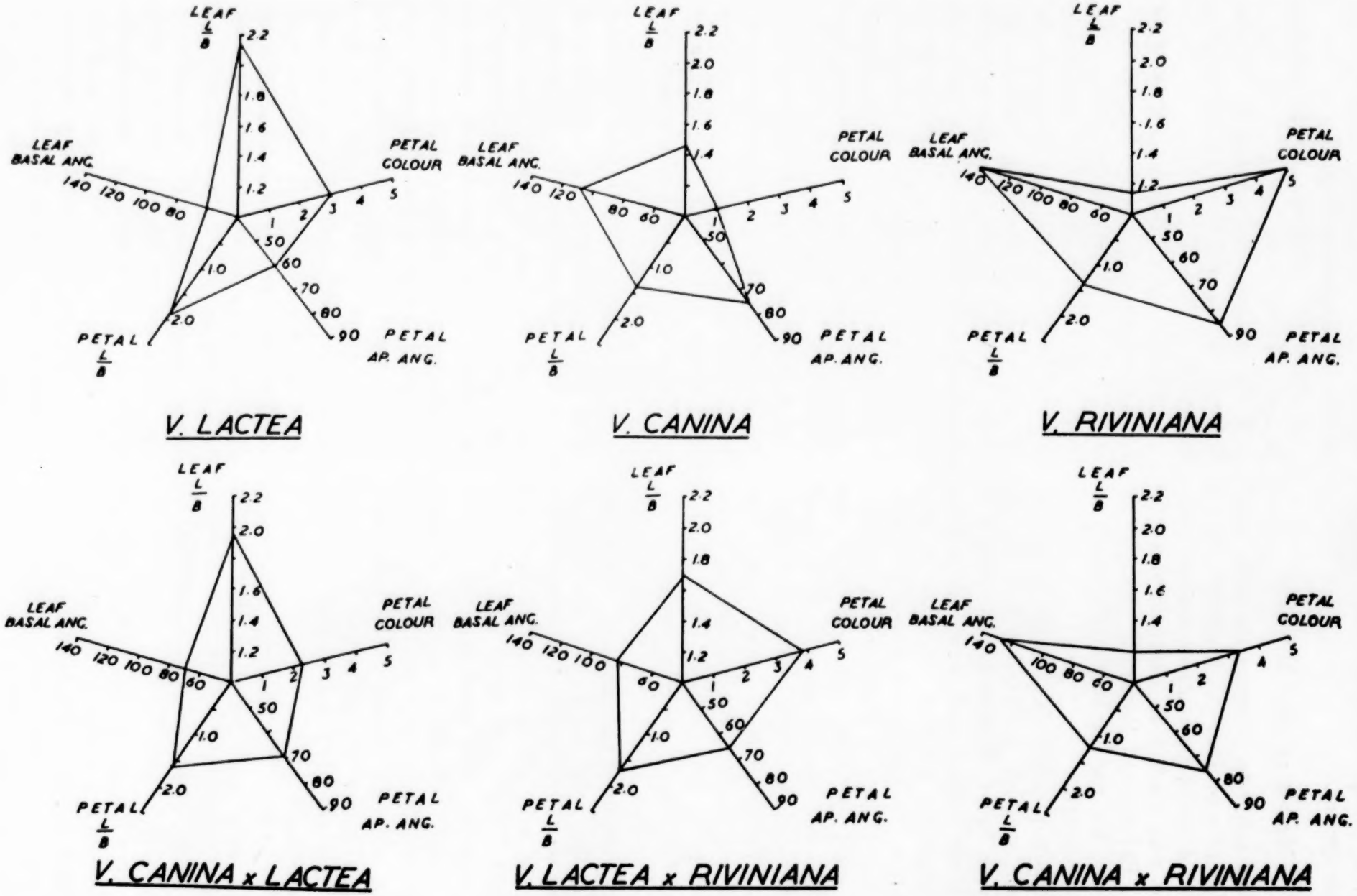


FIG. 1. Cyclic polygons used as standard shapes in the population analyses and drawn from samples of known *Viola* species and hybrids.

(Hutchinson, 1936; Fassett, 1941; Davidson, 1947; Carson and Stalker, 1947) interested in either specific or sub-specific entities. For each sample the means of characters 1–5, listed earlier, are plotted along five axes to give five points which are joined to form a polygonal outline. This polygonal shape is characteristic of the population and speedily shows the affinities between samples and indicates the species involved.

Once the affinities of a population are known, its more detailed composition can be obtained by using pictorialized scatter diagrams (Anderson, 1949) and pollen analyses. The scatter diagrams used here have leaf length/breadth and leaf basal angle as the major axes while, by means of symbols, the petal length/breadth ratios and apical angles of the individual plants are indicated as intermediate or as like one or other of the parent species.

In some cases supplementary information was obtained from the pollen fertility of plants within a sample. One or two anthers were dissected in aceto-carmine and examined under the low power of the microscope; rounded, fully stained grains were considered to be viable. Five random fields were counted for each plant and the results presented as frequency histograms of percentage good pollen (fig. 9).

Results

Space does not allow the presentation of all the data, but the results given in this section are representative. For ease in referring to the code letters of population samples, those involving the three species and hybrids with *V. canina* are designated A–J, while the samples included in the *V. lactea–riviniana* introgression series are numbered 1–10; increase in number reflects decrease of affinities with *V. lactea*. The localities from which the samples, mentioned here, were collected are given in table 2.

Populations comprising species and F_1 hybrids. In figure 1 a set of polygons is presented for samples of known species and F_1 hybrids, both natural and artificial, which serves as a guide to the affinities of all field populations encountered.

The means, and standard errors, of leaf and petal characters for populations of *V. lactea, V. canina* and *V. riviniana* from several areas are given in table 3. Despite their locality and habitat differences the samples for each species are very similar to each other and to those used in the base polygons (fig. 1). These samples show the characteristics of the species, particularly *V. lactea* and *V. riviniana,* in localities where they come into contact and hybridize. On the other hand the general intraspecific variability of each character, which is presented in table 1, is based on collections of material from much of the natural ranges of the three species.

The base polygons for the F_1 hybrids *V. canina* × *lactea* and *V. canina* × *riviniana* were derived from samples of the artificial interspecific crosses growing in the garden; although the latter hybrid often occurs in the field it is included only for completeness and will not be discussed further. The samples of *V. lactea* × *riviniana,* one of which gave the polygon shown in figure 1, were collected in the field from highly floriferous patches of

Table 1. *Intra-specific range, in the three violet species concerned, of the characters based on direct linear measurements*

Species	Leaf		Petal	
	Length/Breadth	Basal angle	Length/Breadth	Apical angle
V. lactea	1.5–2.8	40–75°	1.60–2.50	39–70°
V. canina	1.4–1.7	90–135°	1.00–1.50	65–84°
V. riviniana	0.7–1.3	107–165°	1.14–1.68	75–90°

undoubted hybrid origin showing vegetative proliferation (Valentine, 1949) and characters intermediate between the two species, and were morphologically identical with plants of the F_1 hybrid produced experimentally. Their meiotic cytology was also akin to that of the artificial hybrid.

Wild hybrids intermediate between *V. lactea* and *V. riviniana* were found in most localities visited and one, sample 7, has its scatter diagram illustrated together with

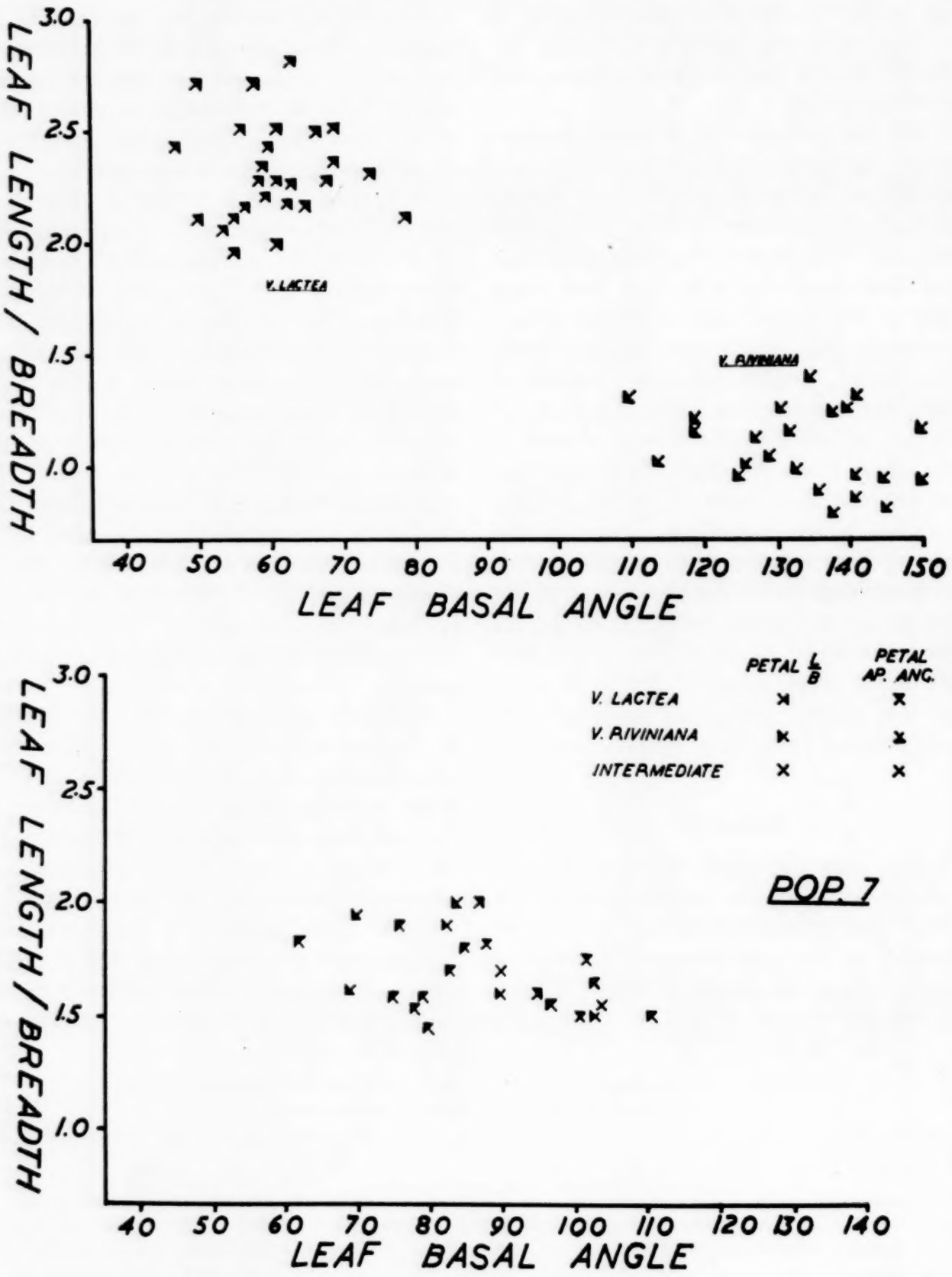


FIG. 2. Scatter diagrams for field population samples of—above, *V. lactea* (A) and *V. riviniana* (G)—below, intermediate hybrid *V. lactea* × *riviniana* (7).

TABLE 2. *Origin of population samples used in the analyses*

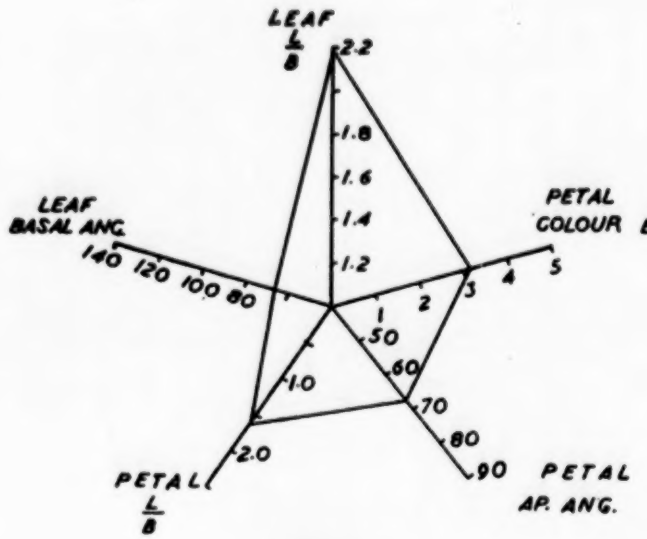
Code of sample	Locality
	V. lactea
A	Gors Goch, Llanbedr Goch, Anglesey, Wales.
B	Roborough Down, nr. Yelverton, S. Devon, England.
C	Serra do Sica, nr. Condeixa, Beira Litoral, Portugal.
	V. canina
D	Piltdown Common, E. Sussex, England.
E	Hooper's Point, Dale, Pembrokeshire, Wales.
F	Dune pasture, Silloth, Cumberland, England.
	V. riviniana
G	Roborough Down, nr. Yelverton, S. Devon, England.
H	Roborough Down, nr. Buckland Monachorum, S. Devon, England.
I	Vale de Canas, nr. Coimbra, Beira Litoral, Portugal.
	V. canina × *lactea*
J	Hooper's Point, Dale, Pembrokeshire, Wales.
	V. lactea ⇄ *riviniana*
1	Bray's Cottage, Goonhilly Downs, E. Cornwall, England.
2	Nr. South Stack, Holy Island, Anglesey, Wales.
3	Valdoeira, nr. Pampilhosa, Beira Litoral, Portugal.
4	Serra da Boa Viagem, nr. Figueira da Foz, Beira Litoral, Portugal.
5	Kynance, Lizard Downs, E. Cornwall, England.
6	Lower Predannack Downs, E. Cornwall, England.
7	Nr. South Stack, Holy Island, Anglesey, Wales.
8	North Common, Chailey, E. Sussex, England.
9	Nr. Porth Towan, E. Cornwall, England.
10	Roborough Down, nr. Buckland Monachorum, S. Devon, England.

those of the parent species (fig. 2). Population 7 occurred as a large floriferous clump, spreading by rhizomes, at the edge of a thicket dominated by *Ulex europaeus* L. and *Rubus fruticosus* L. (agg.) which stood in an area of cliff heath. Hybridization was evidently effected at the junction of typically *riviniana* and *lactea* habitats. The plants (fig. 2) lie midway between the parents on leaf characters while the petals show mixed or intermediate affinities. Sample 8 gave a similar pattern.

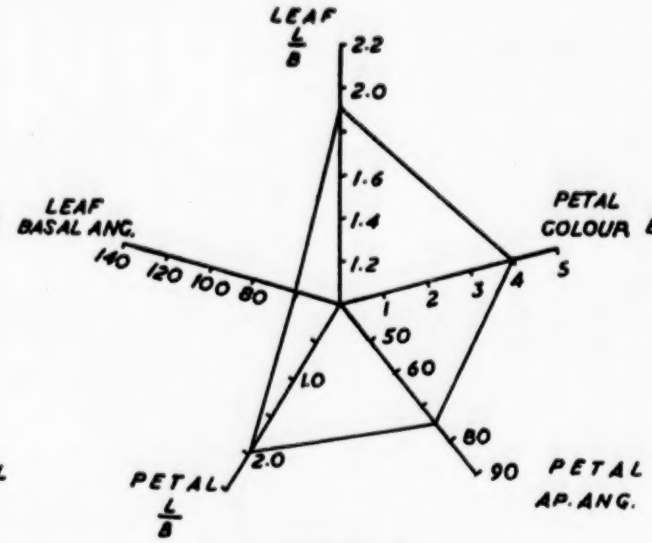
Sample J was taken from the only population discovered of the natural hybrid between *V. lactea* and *V. canina*. The wild hybrid is similar to the F_1 hybrid in cultivation; its characters (table 3) closely resembling those given in the polygon for the artificial population (fig. 1). The habitat had been much disturbed during the construction of a wartime airfield, thus probably accounting for the breakdown of ecological isolation (see Moore, 1958) between *V. lactea,* growing over old red sandstone, and *V. canina,* which was here restricted to the close turfs over boulder clay drift deposits.

In view of the high fertility of this hybrid, the *V. canina* populations flanking the hybrid clumps could be suspected of showing some signs of introgression. Table 3 shows this had probably not occurred, the means and standard errors of the characters being of the same order for samples of *V. canina* (E) found near the hybrid at Hooper's Point and from other localities. Possibly the disturbance of the habitat was too recent to allow extensive hybridization, but it is rather unexpected in view of the facility with which F2 and F3 generations were produced by artificial selfing (Moore, 1957b). A comparable case is described by Stebbins and Ferlan (1956) who found no signs of introgression between *Ophrys lutea* Cav. and *O. fusca* Link. in Algerian populations, despite the apparent fertility of the intermediate hybrids, and their close association with the parents. Similar results for *Primula* are discussed by Clifford (1958) who also cites further examples of a like nature.

Most of the major areas in Britain for *V. lactea* have been visited and *V. canina* is not associated with it anywhere but in



POP. 1



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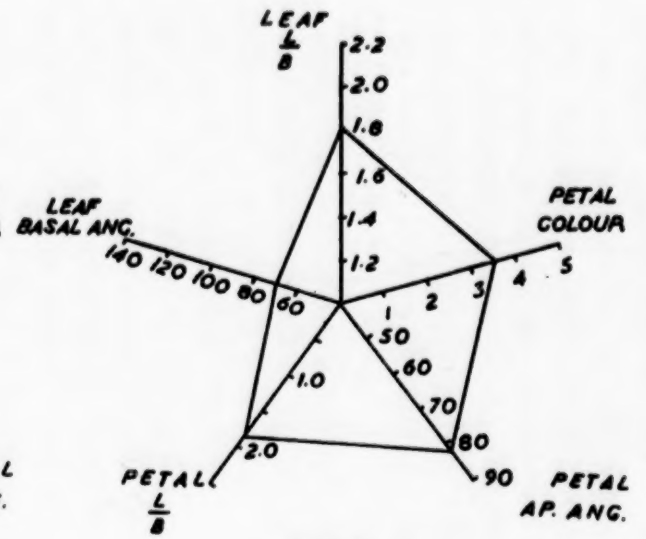

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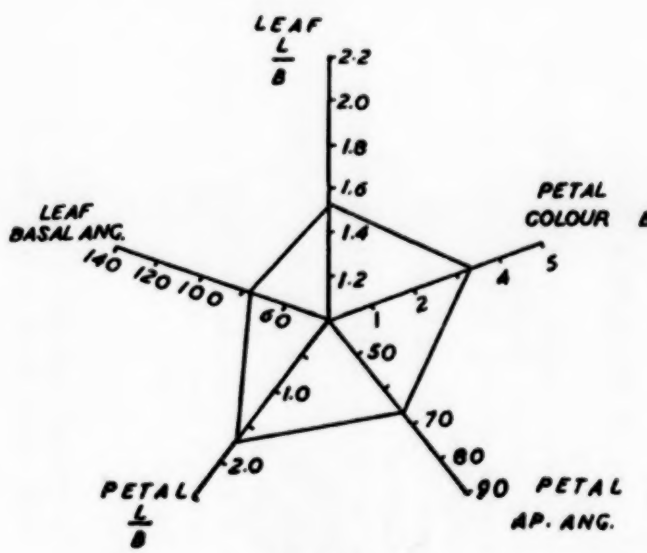

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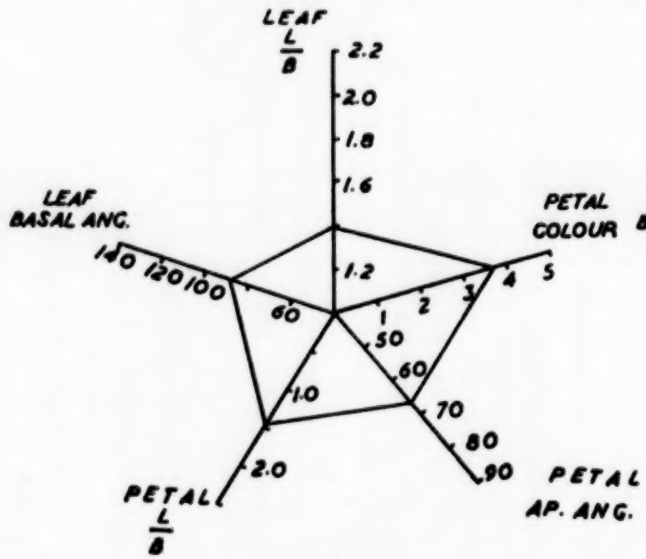

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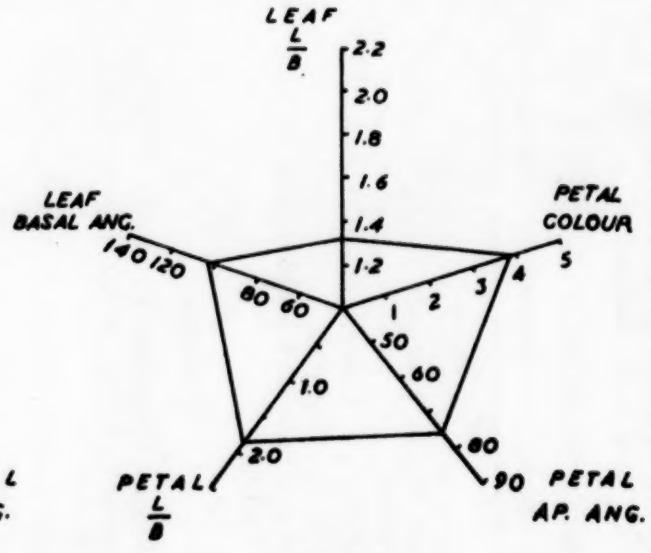

POP. 9

FIG. 3. Cyclic polygons for population samples showing evidence of introgression involving *V. lactea* and *V. riviniana*. A gradation is demonstrated from sample 1, the nearest to *V. lactea*, to sample 9, the nearest to *V. riviniana*.

the one locality mentioned. Field hybridization between these species may be more important than is suggested here but they seem, in general, sufficiently separated by ecological preferences to prevent their crossing.

Introgression involving *V. lactea* and *V. riviniana*

Analysis of field populations. In addition to the intermediate hybrids already described, very many field populations showed varying degrees of influence by *V. lactea* and *V. riviniana* which strongly suggested the existence of introgressive hybridization and some of these populations are used here to indicate the sort of pattern obtained.

The polygons illustrated (fig. 3) are from six representative populations showing the gradual change in shape which reflects the increased influence of *V. riviniana.* Beginning with sample 1, the closest to *V. lactea,* the heightened *riviniana* influence progressively modifies the shape by reducing the leaf length/breadth ratio, increasing the leaf basal angle and petal apical angle, and by changing the petal color index away from the *lactea* value (3) towards that for *riviniana* (5). Sample 6 has a polygon shape near the intermediate hybrid (fig. 1) while sample 9 is still

TABLE 3. *Means and standard errors of leaf and petal characters for each population sample concerned in the analyses*

Code of sample	Leaf			Petal			
	Length (mm.)	Length/Breadth	Basal angle	Color index	Length (mm.)	Length/Breadth	Apical angle
			V. lactea				
A	18.9	2.3 ± 0.05	61.0 ± 1.3	3.0	11.6	1.6 ± 0.05	68.4 ± 1.0
B	15.7	2.3 ± 0.07	53.8 ± 1.4	3.0	11.1	2.0 ± 0.04	59.5 ± 1.0
C	23.0	2.3 ± 0.08	57.1 ± 2.9	3.1	10.9	1.7 ± 0.05	57.0 ± 2.1
			V. canina				
D	11.1	1.4 ± 0.04	109.0 ± 2.2	1.0	9.0	1.4 ± 0.04	82.9 ± 1.2
E	18.3	1.5 ± 0.03	107.9 ± 1.4	1.0	10.3	1.5 ± 0.02	76.3 ± 0.7
F	11.7	1.6 ± 0.03	108.5 ± 1.9	1.0	10.7	1.4 ± 0.04	80.7 ± 0.9
			V. riviniana				
G	9.5	1.1 ± 0.03	134.0 ± 2.9	5.0	8.7	1.4 ± 0.03	93.9 ± 1.0
H	8.4	1.1 ± 0.03	132.9 ± 3.1	5.0	9.1	1.5 ± 0.03	87.9 ± 1.4
I	25.1	1.1 ± 0.02	156.7 ± 4.5	5.0	12.0	1.6 ± 0.07	85.4 ± 1.3
			V. canina × *lactea*				
J	19.0	1.9 ± 0.05	82.5 ± 2.4	1.9	11.1	1.5 ± 0.02.	76.5 ± 0.9
			V. lactea × *riviniana*				
1	21.5	2.2 ± 0.05	66.1 ± 1.4	3.2	12.4	1.6 ± 0.02	67.7 ± 1.0
2	18.2	2.2 ± 0.09	66.2 ± 2.9	4.4	12.5	1.6 ± 0.02	67.1 ± 2.7
3	31.9	1.9 ± 0.06	60.4 ± 1.8	3.9	10.7	2.0 ± 0.01	74.9 ± 0.9
4	33.0	1.8 ± 0.04	70.2 ± 1.8	3.6	13.4	1.8 ± 0.03	82.3 ± 0.6
5	12.4	1.5 ± 0.06	76.0 ± 4.4	3.3	12.2	1.7 ± 0.05	67.3 ± 1.3
6	18.9	1.4 ± 0.04	88.5 ± 3.4	3.8	11.9	1.5 ± 0.04	66.3 ± 1.4
7	23.9	1.7 ± 0.03	101.2 ± 2.0	3.4	12.4	1.5 ± 0.04	69.5 ± 2.7
8	15.4	1.7 ± 0.03	87.2 ± 2.6	4.8	9.9	1.4 ± 0.03	72.5 ± 1.3
9	14.5	1.3 ± 0.03	101.8 ± 2.2	3.9	9.1	1.9 ± 0.04	76.0 ± 2.0
10	7.7	1.2 ± 0.03	132.0 ± 3.7	4.9	7.6	1.6 ± 0.05	66.0 ± 3.6

All measurements were made on plants growing in the wild.
Each measurement refers to samples of 24–30 individuals per population.

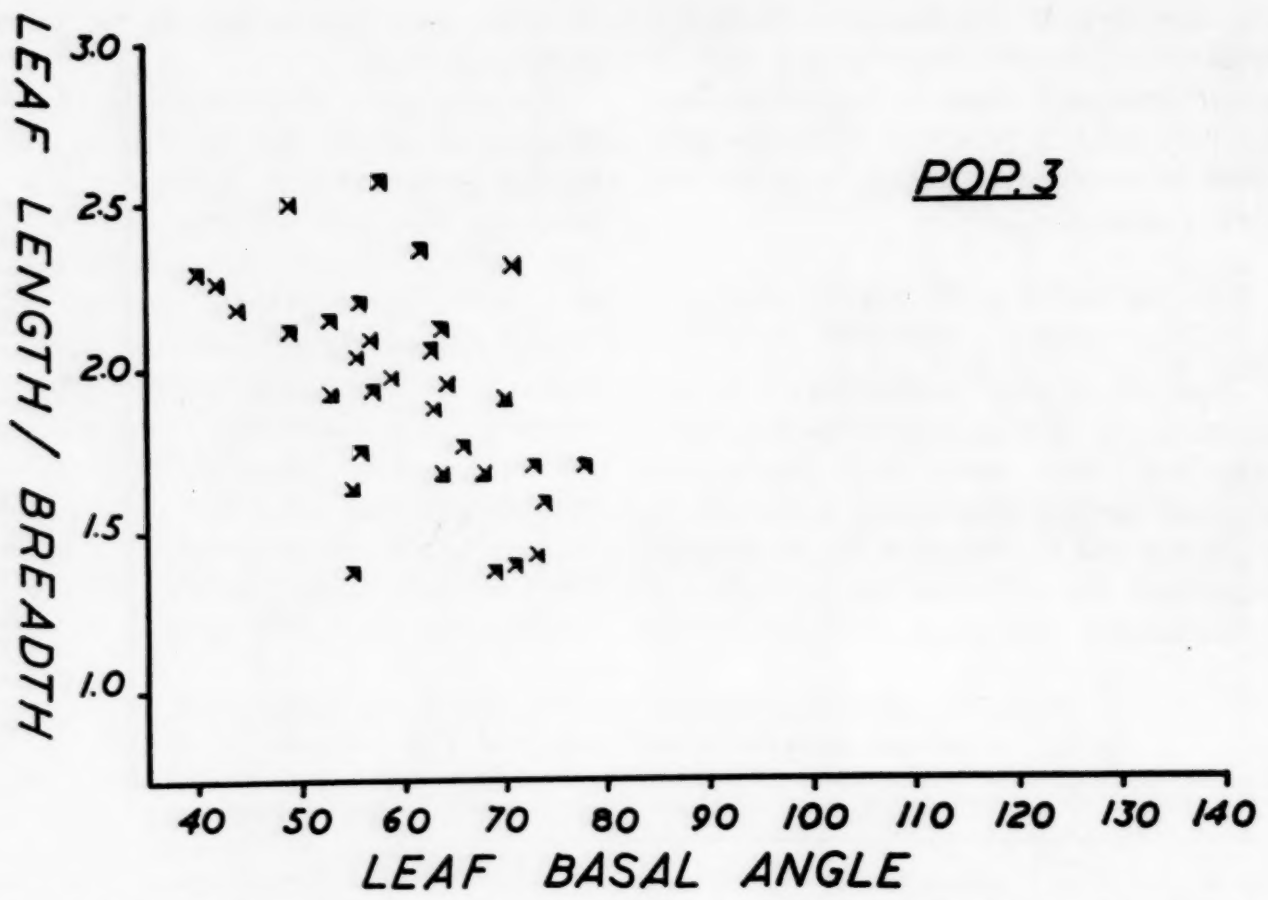

FIG. 4. Scatter diagram for a population sample in the *V. lactea* ⇄ *V. riviniana* introgression series. Sample 3 shows most, and sample 9 (fig. 7) the least, affinities with *V. lactea*.

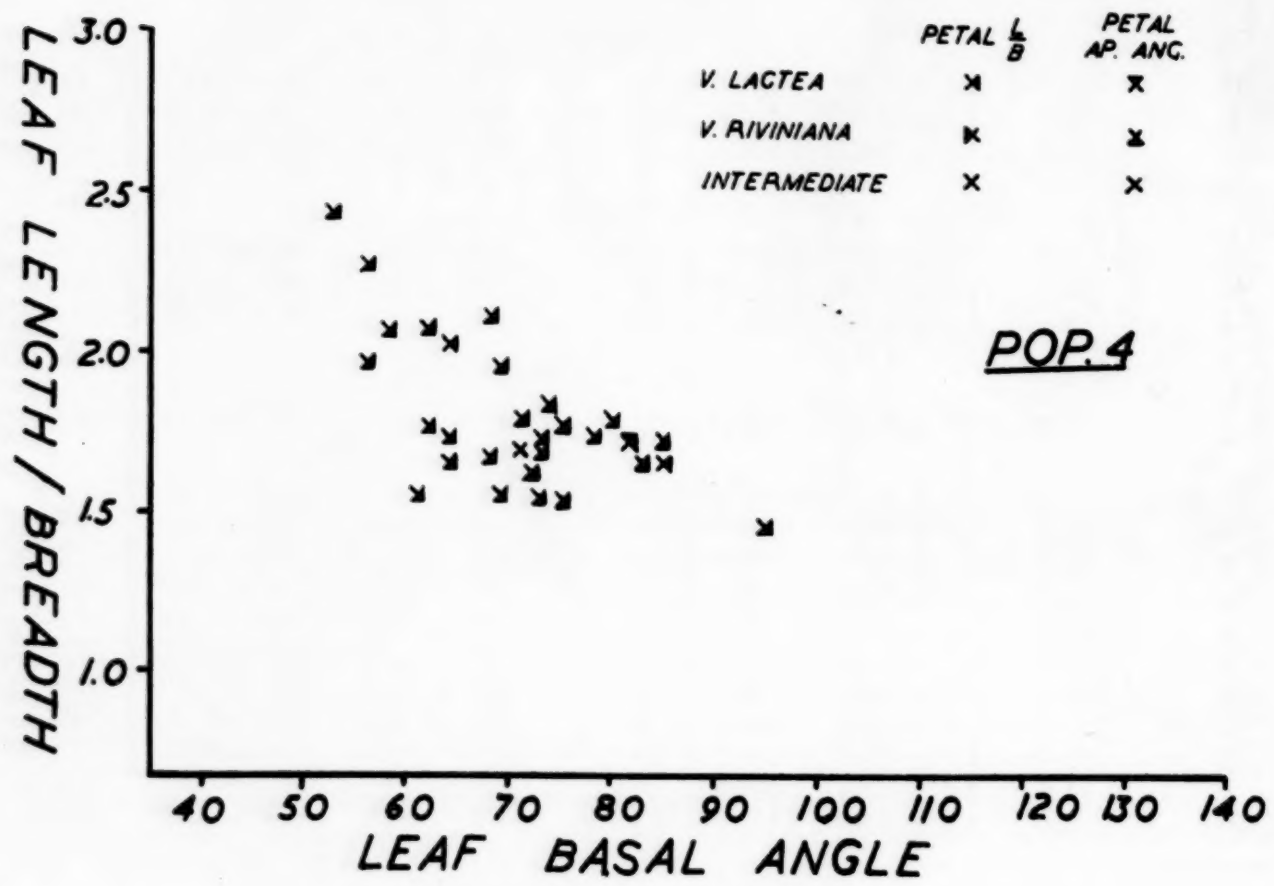

FIG. 5. See caption to figure 4.

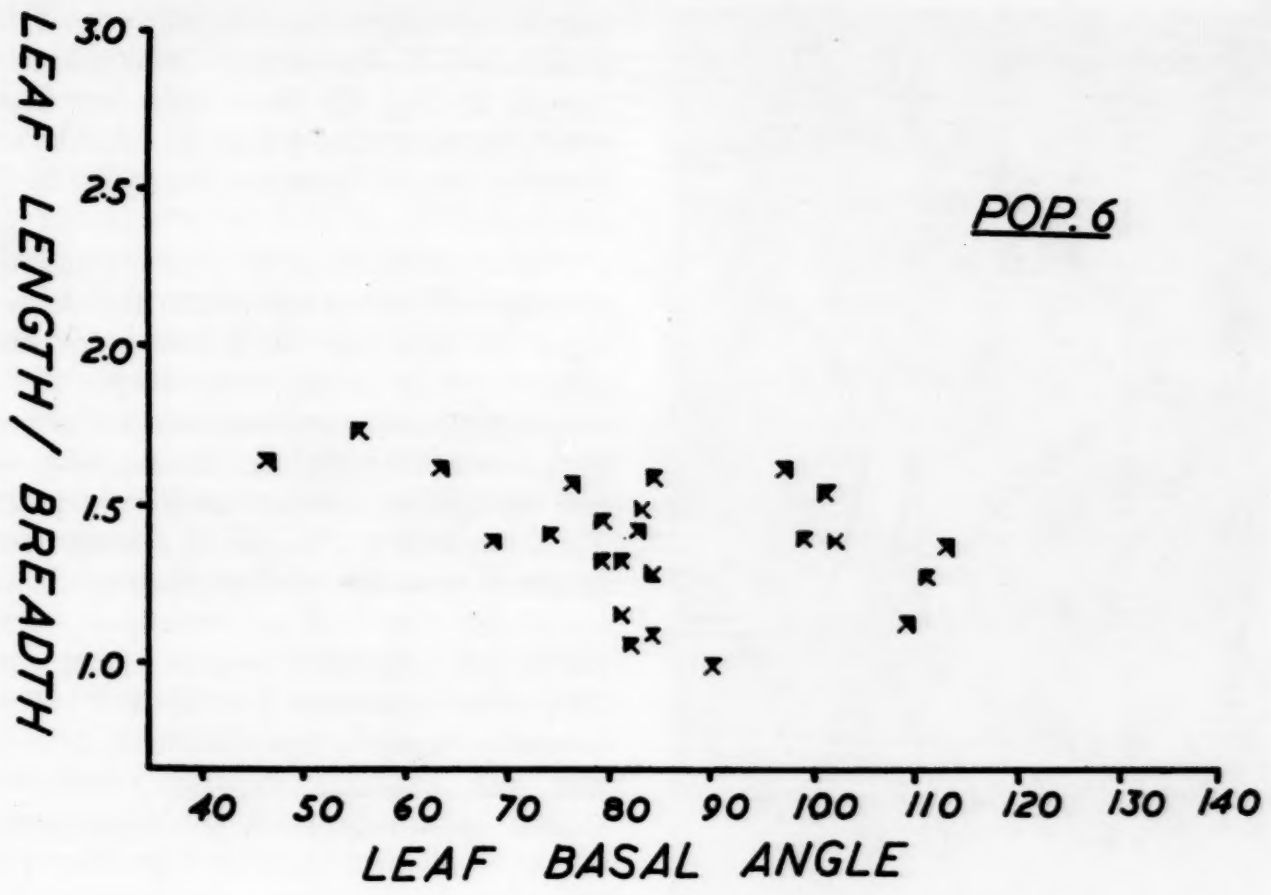

FIG. 6. See caption to figure 4.

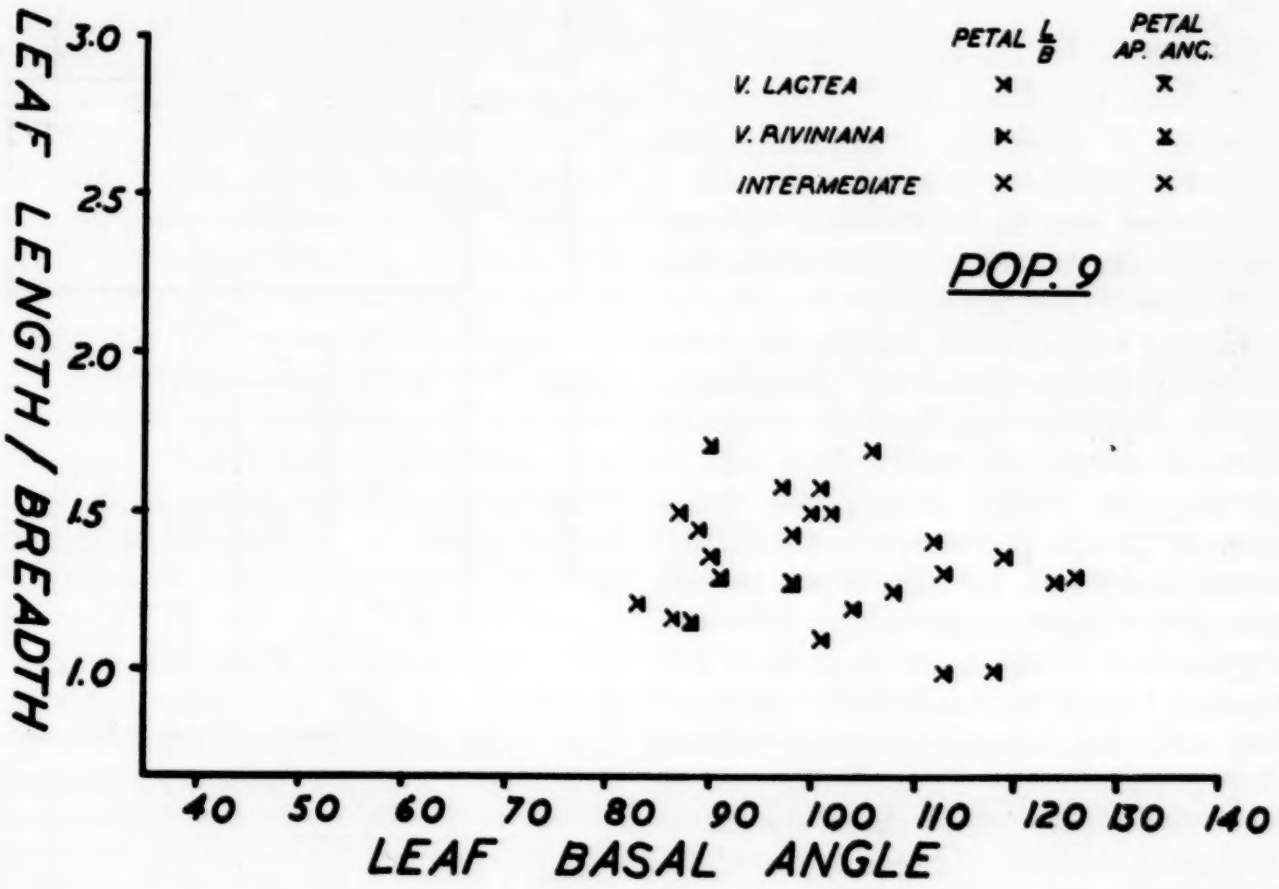

FIG. 7. See caption to figure 4.

FIG. 8. Hybrid between *V. lactea* and *V. riviniana* showing the proliferation by adventitious shoots frequent among such forms. North Common, Chailey, E. Sussex, England. May, 1956.

closer to *V. riviniana*. Another population—10—which is not figured, gave a shape very near to *V. riviniana* but possessed *lactea* influence in the petals (see also table 3).

Having arranged the samples by means of the cyclic polygons the pictorialized scatter diagrams are used to show the changes within the populations accompanying the influx of *riviniana* genes. Sample 1 is like population A (*V. lactea*) in leaf characters but 50% of the individuals have *riviniana* influence in the petals. Figures 4–7 clearly show the general shift towards higher leaf basal angle and lower leaf ratio with approach to *V. riviniana* (e.g. sample G—fig. 2), while the symbols embodied in each point indicate the change in petal characters. Sample 3 (fig. 4) has 30% of plants close to *V. lactea* while samples 4 and 6 (figs. 5 & 6) tend towards the intermediate hybrid (fig. 2) in petal and leaf characters. Most plants in sample 9 (fig. 7) have only one *lactea* petal character and population 10 showed aberrant petal characters in 90% of individuals.

Plants which were morphologically similar to *V. lactea* and which also showed signs of *riviniana* influence were considered to be in the later stages of introgression and they were often observed to possess adventitious shoots (fig. 8) and to grow among thick vegetation. Thus population 1 occurred among dense *Molinia* tussocks and population 3 was associated with a closed community dominated by *Agrostis setacea* Curt. and *Pteridium aquilinum* L. Since *V. lactea* normally occupies open habitats it seems that the physical *riviniana* characters gained by introgression are accompanied by other factors, such as vegetative proliferation and tolerance of competition,

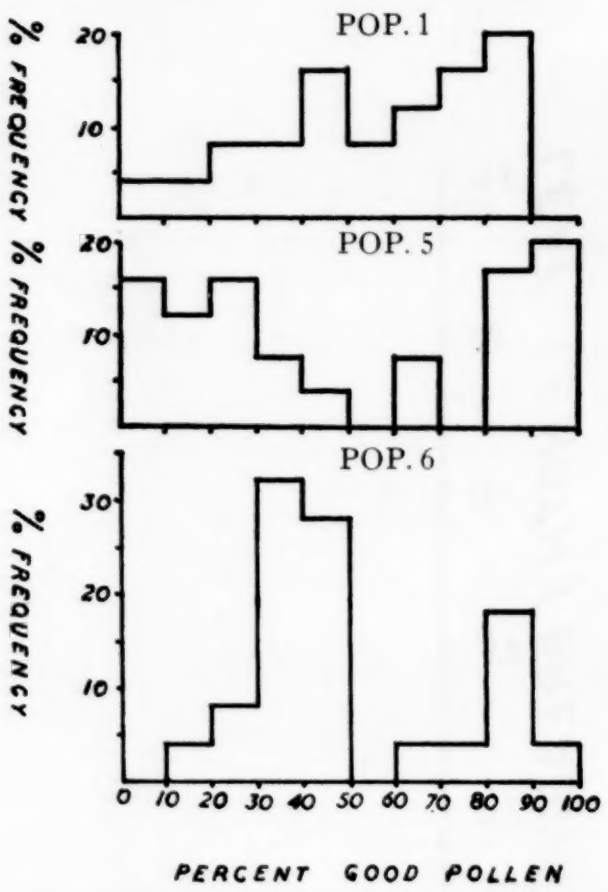

FIG. 9. Percentage frequency distributions for pollen fertility of three population samples in the *V. lactea* ⇄ *V. riviniana* introgression series. Sample 6 has most affinities with the intermediate hybrid and sample 1 is nearest to *V. lactea*.

which are of marked evolutionary importance; the implications of this will be discussed later.

Fertility. The parent species possessed 90–100% good pollen while the artificial F_1 hybrid showed 0–12% pollen fertility. Histograms showing the pollen fertility of three representative samples are presented in figure 9. These data illustrate the general finding that approach to the morphologically parental type is accompanied by rising fertility; some evidence from fruit formation in field populations will be referred to in connection with the later discussion of fertility in these hybrids.

Cytology and artificial crossing. Backcross pollinations were attempted to plants of the intermediate hybrid *V. lactea* × *riviniana* collected in the field; 24 flowers were pollinated from *V. lactea* and 16 from *V. riviniana,* none was successful. Six of these same wild hybrids were kept in the greenhouse for observation over two years; no capsules were formed from "open" flowers while of the many cleistogamous flowers produced by each plant only one yielded a small distorted fruit containing a single fully-formed seed.

In the artificial F_1 hybrid between *V. lactea* (n = 29) and *V. riviniana* (n = 20) the most usual pairing arrangement at first meiotic metaphase is 10 bivalents and 29 univalents (Moore, unpub.). The low fertility of the hybrid may be due to unbalanced gametes resulting from random movement of the univalents. Some field hybrids, morphologically intermediate between *V. lactea* and *V. riviniana,* were collected in population 8 and examined cytologically. Meiosis in pollen mother cells from two plants showed more chromosomes (2n = 50 and 2n = 53) than in the artificial hybrid. This suggests that the few gametes in the F_1 which do prove fertile are those which get a large share of the univalents, and one or two extra probably do not markedly upset the chromosomal balance. This contrasts with the hybrid *V. lactea* × *canina* which loses chromosomes in the F_2 and F_3 generations (Moore, unpub.). One of the wild hybrids, the meiosis of which is considered above, was the plant which selfed to produce a single seed.

Discussion

The methods used enable a ready separation of the species *V. canina, V. lactea* and *V. riviniana,* and their interspecific hybrids. It was possible to deal with all field populations encountered and to obtain a constant and reliable picture from the results.

Despite reports in the literature that the hybrid between *V. lactea* and *V. canina* was not uncommon (Gregory, 1912, and local floras, e.g. Townsend, 1904), and the evidence of its high fertility (Moore, 1957b), only in one case have these two species been found growing together and hybridizing. The population analyses showed all the remaining field hybrids of which *V. lactea* was one parent to be between this species and *V. riviniana,* with which it overlaps ecologically. The many types of hybrid population involving *V. lactea* and *V. riviniana* strongly suggest the occurrence of introgressive hybridization as defined by Anderson (1949), with the main trend being towards *V. lactea.*

Despite the obvious conclusion that backcross pollination of the F_1 hybrid is being effected by one of the parent species and that in this way genes from *V. riviniana* are carried into *V. lactea,* artificial backcrosses were not achieved to either parent and the F_1 hybrid is of low fertility. At this stage the problem is similar to that of Heiser (1951) who reported biometrical evidence of introgression in natural populations of *Helianthus annuus* and *H. debilis* var. *cucumerifolius,* but found their artificial hybrid to be one of very low fertility. Backcrosses to *H. annuus* were unsuccessful but some seeds were produced by open pollination in the field. Similar examples are given for *Geum* by Gajewski (1957) who found the F_1 hybrids *G. montanum* L. × *G. reptans* L. and *G. aleppicum* Jacq. × *G.*

urbanum L. to be highly sterile (0.7 and 0.6% pollen fertility respectively) and yet observed natural introgression between the parent species.

However, these violets are rather specially placed, since in summer and early autumn they produce numbers of cleistogamous flowers, each of which constitutes a chance to achieve self-fertilization. Observations in the greenhouse have indicated that individual F_1 hybrid plants only very occasionally produced any seed by selfing, but several factors must be taken into consideration with this. In almost every locality for *V. lactea* which was visited conspicuous floriferous clumps of the hybrid with *V. riviniana* were to be found; in several cases a single plant had extended vegetatively to cover an area of up to 10 sq.m. These plants bear many more cleistogamous flowers than either parent and seem able to propagate indefinitely by means of adventitious shoots, so that selfing will continue on a considerable scale for many years, and a single initial hybrid may in fact produce much more seed than indicated by the few small greenhouse plants.

With the main sterility barrier of the F_1 hybrid crossed, subsequent plants will probably be more fertile, thus enabling further cleistogamous selfings to occur more readily and backcross pollinations to be effected. Some evidence for this crossing to the parents comes from population 2 which shows every biometrical indication of being intermediate between *V. lactea* and *V. lactea* × *riviniana;* several of the plants when collected were found to possess irregular capsules obviously produced by open pollination since they had the long chasmogamous style still attached to the fruit. It may be noted that the parent species invariably produce well-formed capsules filled with ripe seeds from both open and cleistogamous flowers.

Introgressive hybridization is usually associated with disturbed habitats which enable normally allopatric species to come into contact and provide an open environment in which the hybrids can gain a foothold without the strong competition in the parental communities. Since *V. lactea* characteristically favors open habitats any hybrids produced will be most likely to come into contact with this species. It is thus not surprising to find that much of the introgression is directed towards *V. lactea,* though the reverse process occurs and *V. riviniana* shows *lactea* influence in populations 9 and 10.

Assuming that introgression is taking place, it is interesting to consider the effect which such an influx of genes from *V. riviniana* might have on *V. lactea.* In those populations (1, 2, and 3) postulated as showing the later stages of introgression, it was seen that the plants tended to bear a very close resemblance to *V. lactea* in external characters while possessing additional properties, of which the most important are ability to reproduce vegetatively by means of rhizomes, and the capacity to withstand a good deal of competition from other plants. It has been suggested (Moore, 1957b) that the distribution of *V. lactea* is partly determined by the availability of open habitats whose present open character seems largely due to biotic influences (Moore, 1958), principally to interference with the plant cover by man's activities. A change in the practice of heath burning on British heaths, for example, would cause plant communities to close up and so produce conditions unsuitable to *V. lactea,* while the prevention of erosion by afforestation in Portugal has a similar consequence. In the event of improved pastoralization of its heathy habitats, introgression with *V. riviniana* could be important for the future survival of *V. lactea,* since the additional characters would enable it to adapt itself to the new conditions and to compete on a more equal footing with other species of the closed communities.

In Portugal the more open, heathy habitats, particularly on the higher serras ($>$ 900 m. alt.), carry populations of typical *V. lactea.* At lower altitudes many habitats have been subjected to intense soil erosion which is being checked by

large-scale reafforestation with *Pinus pinaster*. Frequently this has not led to the extinction of *V. lactea* but to the appearance of *riviniana* characters in the populations as has been demonstrated in this account. Population 3 came from this type of habitat and showed marked modification by introgression, since it comprised *lactea*-like plants having *riviniana* influences in some morphological characters (fig. 5), while producing well-formed, fertile capsules.

V. lactea exhibits introgression with *V. riviniana* in populations from both the most northerly and southerly portions of its range and may thus be able to survive under normally adverse environmental conditions. Whatever other factors may later be found to be important it is clear that the future course of variation and evolution in *V. lactea* is likely to be much influenced by the habitats available.

Summary

The occurrence and extent of natural hybridization involving *V. lactea* is assessed by means of population analyses and these data are used in evaluating the field status and evolutionary position of this species. Wild populations including *V. lactea* were sampled over much of its range in Britain and Portugal. Primary classification of populations was by means of polygonal graphs and more detailed analyses were made by using pictorialized scatter diagrams and fertility studies.

The hybrid *V. canina* × *lactea*, though fertile, was found only rarely in the field since the species are ecologically separated and only abnormal habitat conditions bring them together.

The artificial hybrid between *V. lactea* and *V. riviniana* is of low fertility but the species often come into contact in the field and intermediate hybrids are frequent. Results from some population analyses are presented to demonstrate the varying degrees of introgressive hybridization involving *V. lactea* and *V. riviniana*. Some data are given suggesting that introgression is achieved initially by selfing in the cleistogamous flowers of the hybrid and subsequently by outcrossing in later segregates.

The influx of genes from *V. riviniana* has been shown to increase the physiological adaptability of *V. lactea* and the possible implications of this introgression for the future evolution and survival of *V. lactea* are discussed.

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Literature Cited

Anderson, E. 1949. Introgressive Hybridization. New York.

Becker, W. 1910. Violae Europaeae. 1–110. C. Heinrich, Dresden.

Carson, H. L., and H. D. Stalker. 1947. Gene arrangements in natural populations of *Drosophila robusta*. Sturtevant. Evolution, **1**: 113–133.

Clausen, J. 1929. Chromosome number and relationships of N. American species in the genus *Viola*. Ann. Bot., **43**: 741–764.

Clifford, H. T. 1958. Studies in British Primulas. VI. On introgression between Primrose (*Primula vulgaris* Huds.) and Cowslip (*P. veris* L.). New Phyt., **57**: 1–10.

Davidson, J. F. 1947. The polygonal graph for simultaneous portrayal of several variables in population analysis. Madrõno, **9**: 105–110.

FASSETT, N. C. 1941. Mass collections of *Rubus odoratus* and *R. parviflorus*. Ann. Miss. Bot. Gard., **28**: 287–374.

FISHER, R. A. 1936. The use of multiple measurements in taxonomic problems. Ann. Eugenics, **7**: 179–188.

GAJEWSKI, W. 1957. A cytogenetic study on the genus *Geum* L. Monographiae Botanicae, **4**, 415 pp. Warsaw.

GERSHOY, A. 1934. Studies in North American violets. III. Chromosome numbers and species characters. Vt. Agr. Exp. Sta. Bull. 367, 1–91.

GREGORY, E. S. 1912. British Violets. Cambridge.

HEISER, C. B. 1951. Hybridization in the annual sunflowers. *Helianthus annuus* × *H. debilis* var. *cucumerifolius*. Evolution, **5**: 42–51.

HESLOP HARRISON, J. 1949. Field studies in *Orchis* L. 1. The structure of Dactylorchid populations on certain islands in the Inner and Outer Hebrides. Trans. Bot. Soc. Edinb., **35**: 26–66.

HUTCHINSON, A. H. 1936. The polygonal presentation of polyphase phenomena. Trans. Roy. Soc. Canada., (3) **30**(5): 19–26.

MATTHEWS, J. R. 1955. Origin and Distribution of the British Flora. London.

MOORE, D. M. 1957a. The status of *Viola lactea* Sm. in "Progress in the Study of the British Flora" (Bot. Soc. Brit. Isles, London), 97–102.

——. 1957b. The experimental taxonomy of *Viola lactea* Sm. Ph.D. Thesis. University of Durham.

——. 1958. *Viola lactea* (Biological Flora). J. Ecol., **46**(2): 527–535.

RUSSELL, N. H. 1952. An example of introgressive hybridization between *Viola papilionacea* Pursh. and *V. nephrophylla* Greene. Proc. Iowa Acad. Sci., **59**: 134–140.

——. 1954. Three field studies of hybridization in the stemless white violets. Amer. Journ. Bot., **41**: 679–686.

——. 1956. Techniques for species comparison in violets. Proc. Iowa Acad. Sci., **63**: 157–160.

STEBBINS, G. L., AND L. FERLAN. 1956. Population variability, hybridization, and introgression in some species of *Ophrys*. Evolution, **10**: 32–46.

TOWNSEND, F. 1904. Flora of Hampshire, 2nd Edit. London.

VALENTINE, D. H. 1949. Vegetative and cytological variation in *Viola riviniana* Rchb. in "British Flowering Plants and Modern Systematic Methods," 48–53 (Bot. Soc. Brit. Isles, London).

——. 1950. The experimental taxonomy of two species of *Viola*. New Phyt., **49**: 193–212.

WILSON, R. F. 1938. Royal Horticultural Society Colour Chart. British Colour Council, London.

LIMITED PENETRATION OF BARRIERS AS A FACTOR IN EVOLUTION [1]

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Introduction

The purpose of this paper is to present, for discussion and possible future development, an account of the evolutionary stimulus that results from limited penetration of barriers. After several years of study this concept seems to me to be of appreciable importance, but I am uncertain how much novelty can be claimed for it. Some aspects of it are rather obvious; and it clearly impinges to some extent on such familiar evolutionary concepts as differentiation in isolation, quantum evolution, threshold effect, and entrance into new environments. The first of these related concepts has been summarized by Mayr (1954) and the others are reviewed by Simpson (1953). Parts of what I am going to present are perhaps implicit in the writings of some evolutionists and biogeographers, but inadequately emphasized; other parts do not seem to have been considered.

The later drafts of this paper were prepared after the appearance of Darlington's (1957) *Zoogeography*. For information on distribution of the vertebrates I have drawn freely on this source, from which further details may be obtained, but I have not cited every reference to it.

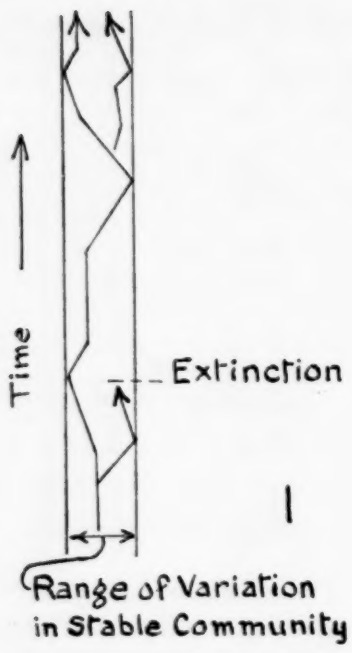
My main thesis is that very restricted and intermittent passage from one region or environment to another provides an evolutionary stimulus to the invader that is lacking if access is free. A corollary for geographic barriers is that exotic diseases introduced with the invader may contribute to the extinction of the original inhabitants of the invaded region. It will be most convenient to demonstrate the process first with geographic barriers.

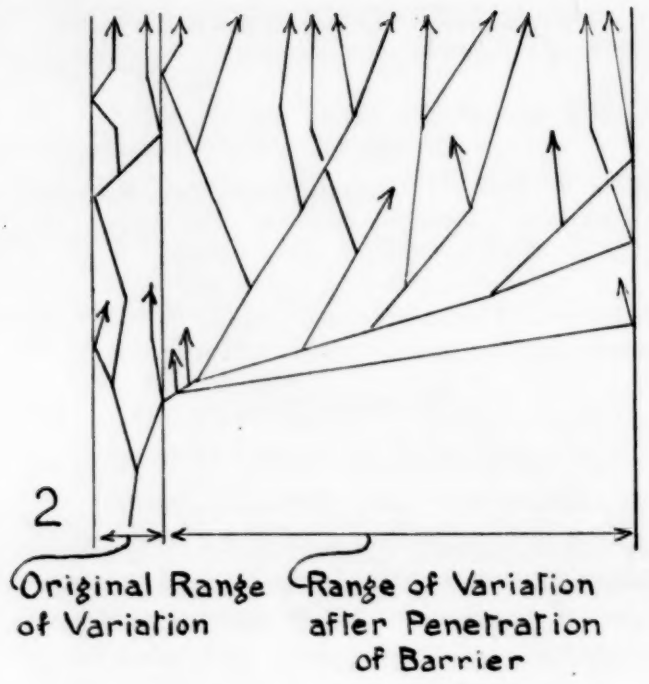
Geographic Barriers

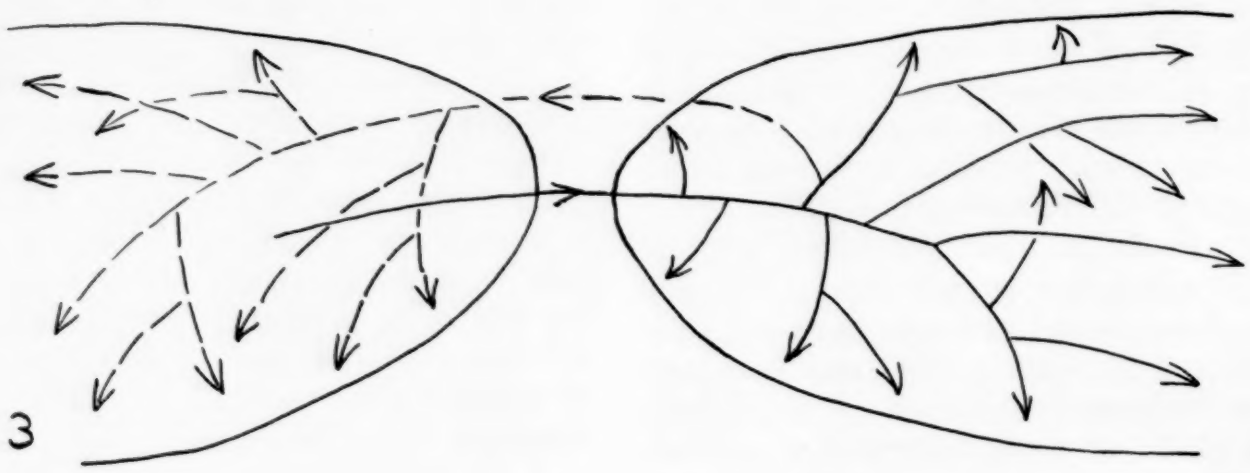
Before proceeding I must explain in what sense I use such phrases as balanced community or relatively stable community in the following discussion, since they have already aroused one critic. In such a general discussion it would be futile to try to define such terms in any precise ecological sense, but I must make it clear that I am not naive enough to suppose that any living community, great or small, ever remains rigidly stable. My instructor in aerodynamics used to introduce the discussion of stability in some such terms as these: if you try to balance a steel ball on a polished steel dome you are faced with instability; if you place it on a flat plate you have static stability, destroyed by the slightest movement; if you bolt it down to the plate you have rigid stability; if you place it in a cup you have dynamic stability—tilting the cup causes the ball to come to rest in a new position. Failure to distinguish these types of stability once moved a biologist of some reputation to deny the reality of any balance of nature. It is, of course, in this sense of dynamic stability that I shall discuss communities with varying degrees of balance.

If the biota of a region moves as a unit it remains essentially a balanced community with all its members subjected to strong and continuous competition. The simplest example of this sort is perhaps a flora reoccupying deglaciated country after the retreat of a continental ice sheet.

[1] Contribution No. 1717 from the Botany and Plant Pathology Division, Science Service, Canada Department of Agriculture, Ottawa, Ontario.







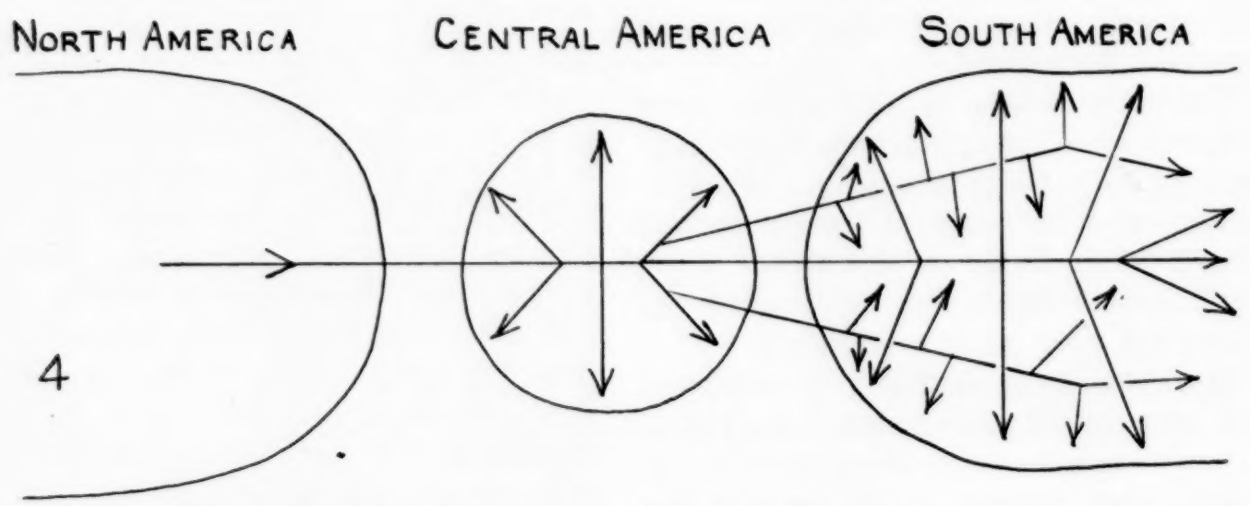


FIG. 1. Limited range of variation possible for organism in stable community with strong competition. Organisms so highly adapted to existing environment that almost any mutation is inadaptive. FIG. 2. Increased range of variation possible when a species penetrates a barrier and is no longer in balance with environment. Some previously inadaptive mutations are now adaptive. FIG. 3. Radiation by organism penetrating from western to eastern continent (solid lines), followed by secondary westward penetration and renewed radiation (broken lines). FIG. 4. Radiation in Tertiary Central America by immigrant from North America, followed by multiple penetration into and radiation in South America. Returning migration and secondary radiation omitted for simplicity.

The plants push forward over a broad front as a relatively stable community. A few casualties occur, some ranges may be distinctly altered, and a few minor novelties develop; but in general the flora is essentially that which was driven out by the ice sheet. Under such conditions of sustained competition, with every species surviving only because it is in good adjustment to its environment, almost every mutation will be deleterious and will be eliminated promptly. We may visualize a species in such a stable environment as developing in a narrow passage between impenetrable walls (fig. 1). Variation is strictly limited. Minor variants do occur, some of which do not survive, but the chances of speciation are slight.

It is rather difficult to visualize two separate continental masses suddenly becoming united across a broad front so that plants and animals could move freely, as communities, from one to the other. If such has ever happened it was probably between Africa and Eurasia when the Tethys Sea diminished; but even here the change must have been rather slow, allowing a gradually increasing passage. If a barrier were quickly and uniformly removed so that two biotas made broad contact, there might be great opportunities for radiation or there might be relatively little. If one biota were dominant over the second it might advance into the second one's territory, causing local or complete extinctions of some species but still moving as a community until halted by novel climatic or other conditions. Despite some exploitation of new niches the total radiation might be small. Gain of territory would be more important than evolutionary gain. A distinctly different situation would occur if only a few species of the first biota were able to penetrate into the territory of the second: the second biota would function as a selective barrier and the conditions would be essentially like those elaborated below.

A very restricted and intermittent connection between continents has been a striking feature of geological history. The Bering Strait region, Beringia in Hultén's (1937) convenient terminology, has at various times provided a land bridge between Eurasia and North America, but movement across it has always been limited by climate in greater and lesser degree and probably by other ecological requirements such as variety of habitat. North and South America were connected at the very start of the Tertiary, and were then separated probably by two narrow sea gaps, until late Pliocene. But the sea barrier was not absolute for some organisms, nor was there unrestricted interchange of plants and animals when the land connection was re-established. The narrow sea gaps certainly allowed limited passage by various birds and plants although generally not by mammals; and the land connection is narrow enough to delay the chance passage of organisms and to exclude some of the habitats that occur to north and south. Wallacea (from Wallace's line eastward to New Guinea) has always been a substantial barrier, limiting exchange between the Oriental and Australian Regions, but it has varied markedly in intensity with fluctuations in sea level. The barrier between Eurasia and Africa is difficult to assess accurately. When the area of the Tethys Sea was greatest it may have been almost absolute for flightless land animals. (Although land plants move on land more slowly than most animals, their ability to cross water gaps seems to be nearly that of land birds.) At present it is essentially a desert barrier, which is highly selective; but during the glaciopluvial ages of the Pleistocene interchange may have been relatively free.

Radiation by animals that penetrate land masses so remote as to be virtually or quite unoccupied by whole orders or classes is a commonplace and scarcely needs mention. Examples are the spectacular radiation of marsupials in the Australian Region, from a very few immigrants; of the Drepaniidae in Hawaii, from a single penetrant species, to 22 species disposed in 9 genera; and of the

Geospizinae in the Galapagos and Cocos Is. into 12 species in 4 genera, again apparently from a single immigrant. But these are cases of radiation against no significant competition, radiation in a sort of biological vacuum. Because there were no other mammals in the Australian Region until a few rodents reached it quite recently, the marsupials radiated without competition into all major mammal niches. Similarly the Hawaiian Drepaniidae and Darwin's finches in the Galapagos radiated without competition into all the major land bird niches. On other isolated islands, such as New Zealand, Madagascar and Mauritius, birds become flightless and invaded vacant essentially mammalian niches.

What we are concerned with in this discussion is the much more frequent radiation into regions in which the major niches were already occupied but not by organisms closely related to the invader. There are innumerable other barriers between continents and islands, and, of course, lesser ecological and physiographic ones within each continent. But the four intercontinental barriers far outweigh the others in significance. Suppose that one of these barriers has been virtually absolute for a period long enough for the biotas on each side of it to become substantially different. On each side of the barrier there will be balanced, relatively stable communities, subject only to minor fluctuations from such causes as climatic change. Now, if an organism manages to penetrate the barrier it enters a community with which it is not in balance, although that community was previously stable. The penetrant may be promptly eliminated; it may exist precariously for some time; or, deprived of its previous checks and in an environment sufficiently novel that a substantial proportion of mutations will be adaptive, it may radiate rapidily and extensively: it is subject both to new checks and to new opportunities. This process is shown diagrammatically in figure 2, in contrast to its condition in the ancestral community.

The difficulty in assessing the importance of this process of limited penetration lies in the fact that major examples of radiation that are suspected of having been stimulated by it are so complex as to defy analysis. It must be demonstrated by simple and inevitably minor examples. A very simple example is seen in the evolution of the Catastomidae, a family of freshwater fishes discussed by Darlington. Most of the family now occurs in North America; but the occurrence of an isolated relict in China distantly related to the other surviving members, indicates that the group originated in Asia, radiated in North America after one representative passed through Beringia, and has recently sent one species back into extreme eastern Asia.

A more complex example, which I have been able to elucidate to a considerable extent, is the family Saxifragaceae, here considered in the restricted sense, excluding such apparently more distantly related plants as *Parnassia, Hydrangea, Ribes,* etc. A little of this information has already been presented (Savile, 1954a), but the details must await a later paper and only a brief summary is presented here. The rust fungi that attack many members of this family can fortunately be arranged, with some reliability, in a fairly complete developmental series, based on a widespread and highly adaptive evolutionary trend that has occurred repeatedly in the genus *Puccinia* (Savile, 1954b). By tabulating the host range of the various rusts we can go far toward arranging the Saxifragaceae in an evolutionary series. It is clear that the early development of the family was in eastern Asia. (The precise area of origin is obscure and would, of course, depend on the arbitrary selection of an original representative of the family even if an adequate fossil record were available.) The most primitive rusts of the family occur between the Himalayas and the Bering Strait. A plant apparently closely ancestral to *Mitella* and *Tiarella,* or perhaps a primitive *Mitella* and some other plant,

crossed via Beringia to the northern Cordillera, where it or they radiated strongly, giving rise to a dozen or so genera that are largely endemic to this region; only *Mitella, Tiarella* and *Heuchera* have reached eastern North America and the first two have spread back into eastern Asia. (*Mitella* may have originated there; it later radiated in Japan.) The exact place of origin of the genus *Saxifraga* is uncertain (again it is largely a matter of definition); but, since the most primitive species show relationship to *Heuchera, Mitella* and *Tiarella,* it appears that *Saxifraga* or its immediate ancestor originated in the northern Cordillera, whence it crossed westward into eastern Asia and radiated substantially. Most of the primitive species of *Saxifraga* occur today in and near Japan, and for practical purposes this region may be regarded as the center of origin for the genus. Even among the relatively primitive sections of the genus several waves of dispersal have occurred and the pattern of rust distribution shows that at least two waves reached back into North America, where the more primitive of two rusts on these plants occurs relatively far south and the more advanced occurs closer to Beringia. Actually some species complexes are centered on Beringia, and several movements across it, with minor radiations, must have occurred from time to time. The more modern species of *Saxifraga* have become increasingly arctic-alpine. The most conspicuous later radiation seems to have occurred first in the Himalayas and finally both there and in the mountains of Europe; but many sections have sent species into the arctic, where dispersal into a holarctic pattern may occur so rapidly and easily (Savile, 1956) that origins become obscure. It should be noted that the contention of Cain (1944), based on the number of species and especially sections in any region, that *Saxifraga* originated in Europe seems to be completely untenable. Darlington points out the danger of basing conclusions on such clues without other supporting data, and this example emphasizes the value of his warning. It must be pointed out that no very primitive and few slightly advanced species of *Saxifraga,* and no species of the genera related to them, occur in Europe. It may also be noted that Cain's figures probably reflect to some extent the distribution of botanists: in such an intensively botanized region as Europe it is likely both that the species have been very completely recorded and that species and sections have been split more finely than elsewhere; at least, than they had been at the time of the last world monograph of *Saxifraga* by Engler and Irmscher (1916–1919). Most of the (very modern) European sections are extremely small and are no better marked than some of the Asiatic species groups into which some of the large sections of Engler and Irmscher are divided. The Bering Strait has been crossed a substantial number of times by some more modern species of *Saxifraga,* in addition to the other crossings indicated above. It was partly from the developing picture of dispersal in the family that I recently (Savile, 1956) emphasized that, for plants, the Bering Strait has been anything but a one-way passage.

It may be useful at this point to use a model to emphasize what we have been discussing. Suppose that an organism passes from west to east across a barrier and sends out six radiating branches like a star-shell. Even after the radial traces fade out the origins of the individual stars may be easily deduced. Let each daughter star now explode to give six more stars that cross each others' tracks; and, once the traces fade out, it becomes impossible to deduce the relationships of the final thirty-six stars. In a radiating group of organisms the situation may be fully as complex as this example, but the pattern will ordinarily be very irregular rather than symmetrical. Figure 3 represents a quite simple radiation (solid lines) subsequent to an eastward penetration; and a similar radiation (broken lines) after a returning westward penetration. A pattern of this modest degree of complexity

might be worked out with considerable accuracy, although it must be noted that adjacent arrowheads are not always closely related. (The diagram can be prepared on the basis of either spatial or phyletic radiation; in the latter situation adjacent arrowheads of diverse origin indicate convergent evolution.) But abundant radiation in the eastern region might well result in not one but several returning westward penetrants. Suppose that, of five such westward penetrants, one became extinct, one persisted without radiating, one radiated slightly and two radiated freely. A detailed interpretation of the situation would be virtually impossible. Given time and persisting favorable conditions some of the new radiants in the western region would again cross the barrier to repeat the process and create a hopelessly confusing picture.

Apart from *Saxifraga,* with over 300 species, the genera of Saxifragaceae are comparatively small. The family is accordingly not a very large one. It shows probably about as complex a pattern of dispersal and evolution as can be elucidated with any reliability. The interpretation of its evolution, which is still far from complete, has consumed a substantial amount of time during more than six years. This fact seems to point a moral. Mass distribution data, if compiled, like those of Darlington for the vertebrates, completely and without bias, are immensely valuable to the biogeographer; but, particularly where biogeographic and evolutionary studies are to be combined, we must have a few fully developed examples to complement the purely statistical data. A complex example cannot be resolved by the mere accumulation of published data, but requires prolonged personal study. It is clear that few such examples can be analyzed and that they should be chosen carefully if wasted effort is to be avoided. In picking examples it thus seems advisable to select groups of organisms that give promise of complementary attacks through several disciplines. I did not, of course, select the Saxifragaceae with any such prescience. I was forced by an accumulation of specimens to revise its parasites, and only then did I see any promise in an evolutionary study of the family. It was in the development of the study that the concept being presented in this paper began to emerge.

How frequent has this radiation after penetration been? The question can never be answered with exactness, but it would be an odd chance if this family that I was led to study were a rare exception. When we consider the four major intercontinental and many lesser barriers, it is reasonable to suppose that examples fully as important as the Saxifragaceae have occurred many times only to be obscured by their own complexity or by extinctions and an inadequate fossil record.

With this background it may be well to consider briefly the immensely rich bird fauna of Central and South America. South America was separated from North America for almost all the Tertiary. During this prolonged isolation the marsupial mammals that reached South America before the connection was broken radiated quite strongly. Later, presumably at times when the sea gaps were reduced by islands, there were limited invasions by groups of mammals, "island-hoppers" in the terminology of Simpson (1951), with some ability to cross water barriers. Finally, in late Pliocene, placental mammals streamed in from North America, eliminating most of the marsupials and radiating in turn. We are left with a mammal fauna that, although it is quite rich and shows considerable endemism, is much less complex than the bird fauna, and whose evolution is simple enough to be traced in broad outline.

The situation was far more complex for birds than for mammals. The Tertiary barrier between North and Soutk America was never complete for birds. Actually there appear to have been at times two sea barriers, each narrow enough to be crossed occasionally by land birds, with an island between them that

may, for our present purpose, be somewhat inexactly called Central America. Radiation after limited penetration in such a situation presents a more complex picture than we have yet visualized (fig. 4). If an organism crosses from North to Central America, after the latter has been isolated for some time, it may radiate there. One to several of the resulting species may penetrate to South America to radiate again. Figure 4 shows the beginning of such a process in very simplified form with no diversification of the original radiants. If we postulate (a) such diversification, (b) return of Central American radiants to North America and radiation there, (c) returns from South to Central America with renewed radiation, (d) penetration north and south by these new Central American radiants, and (e) intermittent extinctions of some groups in each of the three regions, the complexity of the pattern becomes immense. Yet this may be considerably simpler than the real pattern of bird evolution near these Tertiary water gaps. Of the three regions South America has much the richest bird fauna. North America is large but is temperate; only its southern fringe is subtropical. Central America is tropical but relatively small. South America is large and much of it is tropical: fed by intermittent immigrants from the north the development there of a rich bird fauna was perhaps inevitable. It may be noted that South America is particularly rich in the rather modern suboscine passerines, which is in accord with a Tertiary stimulus to evolution.

The question of a presumptive Tertiary tropical North American bird fauna is related to that of the evolution of South American birds. Darlington summarizes the problem in the latter part of his chapter on birds and concludes that the sum of evidence is against such a tropical fauna. Darlington's conclusions seem to be sound, and I simply wish to point out, in support, that the process which I have outlined emphasizes the danger of assuming that any group currently close to one of the Central American barriers originated on the side where its species now predominate.

Darlington cites large area and tropical climate as the reasons for the rich fauna of the Oriental Region. To these causes I would add the intermittent isolation, to variable degrees, of Sumatra, Borneo and the smaller Indonesian islands. As far as birds are concerned New Guinea and the Philippines may perhaps be added to the list.

Ecological Barriers

It goes without saying that when organisms penetrate into a hitherto vacant niche they commonly radiate vigorously. We also know that substantial radiation may follow exploitation of occupied niches, as when various terrestrial vertebrates have reverted to aquatic habitats. Again the question arises as to whether limited penetration of the barrier may be a greater evolutionary stimulus than general penetration. The question is perhaps an academic one in this case, because it is doubtful whether anything but limited penetration has ever occurred. The principle probably still holds; for if, for example, numerous algae had moved more or less simultaneously from aquatic to marshy land habitats to initiate a land flora the opportunities for radiation would have been restricted by competition between the migrants.

Such changes as from aquatic to terrestrial habitats are gradual and thus are not fully comparable to the penetration of geographic barriers; but it is probable that some ecological barriers are penetrated much more abruptly. One example in the fungi, which I have already elaborated (Savile, 1955), concerns the origin of the Basidiomycetes. It appears that the class originated from a primitive Ascomycete akin to the modern genus *Taph*- with water, and are dispersed in water. Occasionally, but aberrantly, *Taphrina* bears its spores externally on delicate projections from the ascus wall. In the Basidiomycetes the spores are regularly

borne externally on similar projections (sterigmata) formed on the basidia, which are homologous with the asci; but, as an additional refinement, the spores are forcibly discharged by the turgor of the basidium. By this change in discharge mechanism the Basidiomycetes were enabled to discharge their spores in moist air rather than water, and the spores dispersed in air instead of water. The class thus became quite abruptly adapted to habitats and climatic conditions quite different from those to which their ascomycetous ancestors were best suited. Refinement of the spore discharge mechanism presumably needed a number of mutations; but an early mutation marked the crossing of a threshold into the new environment and started the elaboration of a very large and successful class of organisms. It may be added that in the early stage of their evolution the Basidiomycetes seem to have been subject to little competition; but later they and the Ascomycetes, by various refinements, became able to compete for several niches.

A reviewer has objected to the suggestion, made in the preceding paragraph, that a single simple mutation may be adaptive, and, particularly, that a single-gene mutation may be so. Not being a geneticist I shall not attempt to deal quantitatively with this objection; in fact I doubt whether the data for such a treatment exist. However, I can deal with it qualitatively by example. An excellent example in support of my suggestion has become available with the opportune appearance of the demonstration by Mangelsdorf (1958) that, in plants of certain genetic combinations synthesized by him from primitive varieties of corn (*Zea mays*), the pod-corn gene tips the scale between adaptation to survival in the wild state and adaptation to survival as a cultivated plant. Plants possessing this gene bear most of their seeds in free florets that dehisce at maturity to allow dispersal. Plants without it bear the seeds, without enclosing glumes, in ears that are readily harvested and processed, but which make the plant inadaptive to natural dispersal and thus incapable of survival in the wild state. The remarkable synthesis seems to go far toward solving the mystery of the origin of the corn plant. It is difficult to imagine a more elegant example of an evolutionary threshold. The new ecological niche in this instance is cultivation, a symbiosis between man and the crop plant. The threshold must seldom, I imagine, be so spectacularly conspicuous; but by definition it is a point on an evolutionary sequence rather than a large part of the sequence.

The objection further infers that, when a structure or mechanism is developed by a series of mutations, the whole series may be adaptive but none of its component parts can be; i.e. that the whole is necessarily greater than the sum of its component parts. It is conceivable that occasionally a set of individually useless mutations may combine to produce a useful character, but most of the useless mutations are likely to be eliminated before the "winning combination" can be completed. In all the adaptive structures whose evolution I have studied (such as sculptured spore walls of fungi; barbed, hooked, plumed or winged seeds and fruits; and bill and flight feather modifications in birds) gradually increasing degrees of adaptation are to be seen. It is clear that the individual changes are adaptive in such a series.

Extinction

So far we have considered only the positive evolutionary effects of barrier penetration—radiation. We must also consider the negative effects—extinction. Extinction is a general counterpart of radiation or range extension, of whatever origin. But there is one cause of extinction that is peculiarly the result of intermittent contact between continents or smaller land masses, namely the introduction of exotic diseases. The potentiality of such diseases as a factor in extinction will perhaps be more evident to plant pathologists

and quarantine officials than to most students of evolution. When two regions are effectively isolated for a prolonged period the populations of each tend to develop resistance or tolerance to their own diseases; but they often become highly susceptible to disease of related populations in the other region against which there has been no selective pressure. Our information on this point comes largely from diseases introduced accidentally by man, but the principle should apply equally well to natural introductions. Several examples can be cited from plant diseases. (a) The fungus *Endothia parasitica* occurs naturally on the Asiatic chestnut, *Castanea mollissima,* in China, causing minor twig cankers. It was introduced, unnoticed, into New York with this tree, as nearly as the story can be reconstructed, and promptly attacked the American chestnut, *C. dentata,* with such vigor that within about twenty years all the major stands of this important tree were virtually wiped out. It has more recently reached Europe and has destroyed many stands of *C. sativa.* (b) The story of the mis-named Dutch elm disease, caused by *Ceratostomella ulmi,* is very similar. It was evidently introduced into Europe with resistant Asiatic elms (*Ulmus* spp.), and soon took a heavy toll of the native species. From Europe it was twice introduced into North America, apparently once with infected logs and once with crating wood, and rapidly made severe inroads into the American elms although there seems to be hope of resistant residues being left. (c) *Cryptostroma corticale* has been known for years as an inconspicuous, apparently saprophytic fungus on the bark of fallen maples in the Great Lakes region of North America. It seems to have been introduced with timber into dock areas east of London, England. In 1945 it was found attacking a tree of the sycamore maple, *Acer pseudoplatanus,* in nearby Wanstead Park. Five years later it was attacking nearby stands and estimated to be killing 15–20% of trees and saplings in a stand per annum (Gregory and Waller, 1951) and by 1955 it had reached other parts of England and continental Europe.

The occurrence of a barrier within Eurasia should be noted in connection with examples (a) and (b). Under Pleistocene and Recent conditions *Castanea,* a warm-temperate genus, has been unable to traverse central Asia and is absent from the Causcasus to western China. For *Ulmus,* a more northern genus, the barrier is not so wide. A few species do occur in central Asia, but they are separated from the European species by the Ural Mts., the Caspian Sea and the arid area to the east of them.

Usually an introduced pathogen will not completely destroy a native susceptible host species unless a tolerant host is also present or the pathogen can exist saprophytically, for the parasite cannot maintain itself if the host population falls below a critical density—a common limitation in the use of biological control measures. But if a tolerant or resistant host invades the region along with the pathogen it may both compete for the habitat of the native host and maintain a reservoir of inoculum. Under such conditions an introduced disease may well exterminate its host or so reduce its numbers that it dies out from other causes.

The examples that I have given refer to plant diseases with which I am familiar, but it would be surprising if the phenomenon never occurred with animal diseases. I had thought that the virtual disappearance of the native squirrel, *Sciurus vulgaris leucurus,* from southern England nearly concurrently with the introduction of the American grey squirrel, *S. carolinensis,* might constitute such an example. I am obliged to Dr. Maurice Burton for directing my attention to the discussion of the problem by Shorten (1954). An introduced disease does seem to have played a part in the decline of the native squirrel, but it was evidently introduced before the release of the grey squirrel and its source is obscure.

It should be noted that extinctions

through disease might operate between organisms with almost any degree of taxonomic difference from subspecies upward. Most fungal and bacterial pathogens of plants are quite closely restricted in host range; but many viruses spread across families, orders or even subclasses. Cases are known among cultivated plants where all individuals of one species are infected by a virus but suffer little damage, and another species, grown nearby, is also nearly 100% infected and the plants are killed outright or so weakened that they produce no seed. The potentiality for destruction of such a susceptible species growing in the same habitat as a tolerant one is obvious. Among animal diseases the viruses vary greatly in host range. Some of the avian viruses (ornithoses) are highly virulent to man, although fortunately infection does not seem to occur very easily.

The period of isolation needed for a population to become susceptible to a disease need not be very long, perhaps only a few thousand years. The susceptibility of the Eskimo and American Indian to diseases brought to North America by the white man has been a subject of frequent comment. Although some of this susceptibility is unquestionably due to environment much of it seems to be genetic. In the disastrous outbreak of poliomyelitis at Chesterfield Inlet in 1949 it was found by Dr. J. P. Moody (personal communication) that some Eskimos took the disease twice, which suggests an almost complete inability to produce antibodies against the virus, only one strain of which seems to have been involved.

Extinctions are commonly explained by large-scale climatic changes, extreme orographic activity, competition and predation. Climatic changes may have been partly responsible for the disappearance of the giant reptiles at the end of the Cretaceous. Orographic activity, apart from extremely localized disasters, probably operates chiefly in the modification of climate. Competition and predation were probably mainly responsible for the extinction of most of the South American marsupials and perhaps for various other large-scale extinctions. Without wishing to make any exorbitant claims for the part played by introduced disease in extinction, I feel it must be ranked with these other factors. Three well-documented families of mammals come to mind as possible examples of extinction by disease in North American. These are Tapiridae, Camelidae and Equidae, all of which underwent much of their evolution in North America but now persist only in the peripheral parts of their former ranges. They became extinct in North America respectively in late Pleistocene, the extreme end of the Pleistocene, and post-Pleistocene. It has been suggested to me that a probable cause of extinction, at least for the last-named family, was human predation. This factor must, of course, be considered, but I find it difficult to believe that, with his primitive equipment and probably small population at the time involved, early man would have made very serious inroads into a population not already reduced by other means. The lowered Pleistocene sea levels and the accompanying climatic changes in central America could have facilitated the introduction of various diseases.

The possibility of introduced diseases being a cause of abrupt animal extinctions has, not unnaturally, met with varied reactions; and one or two people have condemned it bitterly. I certainly make no lavish claims for it; but we have many well-documented examples of the way in which the mechanism operates in plant diseases, in addition to those already cited; plants and animals do live in the same world, subject to the same changes in world conditions and showing many similar biogeopraphic patterns; and, although some of the details of epidemiology are very different in the two kingdoms, most of the principles are common to both. It would be inadvisable to assume that this mechanism does not operate in animals merely because well-documented examples

of its operation are not immediately forthcoming.

Summary

If two adjacent land masses are effectively separated for prolonged periods each acquires a relatively stable biota, but the two biotas will be appreciably different. The organisms in each will be subject to strong and constant competition and all will be highly adapted to the existing conditions. Consequently almost all mutations will be deleterious and evolution will be restricted. If an organism manages to cross the barrier to the other continent, and to survive, it will no longer be in balance with the community, some of its mutations will be newly adaptive, and it may radiate vigorously. Some of the radiants may return across the barrier and radiate again. Consequently it may be impossible to deduce the area in which a group originated. A somewhat similar process may operate when ecological barriers are penetrated. If many organisms cross a barrier nearly simultaneously the competition remains more intense and the chance of any one of them radiating strongly is reduced. Organisms in an isolated continent are not subject to selection for resistance to diseases that occur in other continents and are frequently highly susceptible to them. Occasional intercontinental connections may result in the introduction of exotic diseases and of tolerant or resistant organisms to carry them, which may explain some of the abrupt extinctions recorded by the paleontologist.

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Literature Cited

Cain, S. A. 1944. Foundations of plant geography. Harper & Bros., New York.

Darlington, P. J., Jr. 1957. Zoogeography: the geographic distribution of animals. John Wiley & Sons, New York.

Engler, A., and E. Irmscher. 1916–1919. *Saxifraga*. In Das Pflanzenreich, ed. A. Engler, IV, 117, Heft 67, 69.

Gregory, P. H., and S. Waller. 1951. *Cryptostroma corticale* and sooty bark disease of sycamore (*Acer pseudoplatanus*). Trans. Brit. Mycol. Soc. **34**: 579–597.

Hultén, E. 1937. Outline of the history of arctic and boreal biota during the Quaternary Period. Bokförlags Aktiebolaget Thule, Stockholm.

Mangelsdorf, P. C. 1958. Ancestor of corn. Science, **128**: 1313–1320.

Mayr, E. 1954. Change of genetic environment and evolution. In Evolution as a process, ed. J. Huxley, A. C. Hardy and E. B. Ford. Geo. Allen & Unwin, London.

Ridley, H. N. 1930. The dispersal of plants throughout the world. L. Reeve & Co., Ashford, Kent.

Savile, D. B. O. 1954a. Taxonomy, phylogeny, host relationship and phytogeography of the microcyclic rusts of Saxifragaceae. Can. Journ. Bot. **32**: 400–425.

——. 1954b. Cellular mechanics, taxonomy and evolution in the Uredinales and Ustilaginales. Mycologia **46**: 736–761.

——. 1955. A phylogeny of the Basidiomycetes. Can. Journ. Bot., **33**: 60–104.

——. 1956. Known dispersal rates and migratory potentials as clues to the origin of the North American Biota. Amer. Midl. Nat. **56**: 434–453.

Shorten, M. 1954. Squirrels. Collins, London.

Simpson, G. G. 1951. History of the fauna of Latin America. In Science in progress, seventh series, ed. G. A. Baitsell. Yale University Press, New Haven.

——. 1953. The major features of evolution. Columbia Univ. Press, New York.

THE EVOLUTION OF MAMMALIAN CHARACTERS

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INTRODUCTION

The problems of the origin of mammals have been under investigation for about one hundred years by comparative anatomists and paleontologists. Today, we can affirm with considerable confidence that the mammals arose from therapsid reptiles whose origins trace through pelycosaurian ancestors to the captorhinomorphs. The general patterns of morphological change are well understood, so far as osteology is concerned, and some attention has been given to inferences of the evolution of soft anatomical systems.

Interest in the origin of mammals has been renewed in recent years by new materials and restudy of older materials. Recent new finds in the Russian Permian along with restudies of older materials from beds of this age, and recent finds in the lower mid-Permian of Texas have added to our earlier picture the concept of faunal ecology in evolution. Efremov (1950, 1954, 1955) has stressed the distinctions between what he terms "archaic" and "advanced" faunas. The former represent modified persistent faunas of the Carboniferous type which maintained themselves on lowlands, primarily deltas. "Advanced" faunas, which in the present case are dominated by therapsid reptiles, have developed in the course of evolution on the continental areas away from the marginal lowlands and deltas. The San Angelo and Flower Pot formations, early mid-Permian of Texas (Olson and Beerbower, 1953), exhibit these two types in stratigraphic succession with "archaic" faunas in the mid-San Angelo and basal Flower Pot red shales, and an "advanced" fauna in the coarser clastics of the upper San Angelo. These beds are essentially contemporary with zones 1 and 2 of the Russian "Upper" Permian, but in the latter the two faunal types occur contemporaneously under different conditions of deposition. We know of no "advanced" elements in the lower Permian, apparently because the beds that carry fossils were formed only in very low lands and largely in deltaic areas. It is also important to realize that some elements were common to both types of faunas, especially certain captorhinids and sphenacodont pelycosaurs. Faunal boundaries, thus, are by no means absolute and it is the total composition of the faunal complexes and not one or another genus or family that is essential to its recognition.

With respect to new interpretations of the course of evolution, Brink (1956) has outlined a series of challenging speculations upon the state of body temperature, respiration and reproduction in the therapsids, with specific reference to the cynodonts. He cites, in rib structure, evidence for the existence of a diaphragm. Other items, the secondary palate, ethmoid turbinals, evidence for the existence of "vibrissae," and evidence of glands in the snout are given in support of his contention that a mammal-like physiology was initiated early. On the basis of dentition, the form, arrangement and replacement of teeth, he speculates further upon the origin of the mammalian type of birth. While highly conjectural, his remarks cannot fail to spark new lines of thought on the problems of mammalian origins.

New materials of very advanced therapsids and very early mammals are contributing importantly to knowledge of the critical intermediate reptilian-mammalian stage of development. Crompton (1958)

has described an "ictidosaur," which he has named *Diarthrognathus,* and has emphasized the existence of a double articulation of the skull and lower jaw, involving both reptilian and mammalian elements. A recent publication of Kühne (1956) greatly increases the knowledge of the bienotheres by a detailed analysis of *Oligokyphus.* Recently, as well, I have been loaned by Father Harold Rigney a wealth of specimens of *Bienotherium* from the lower part of the LuFeng series in Yunnan, China. These are under study, as are the rather startling finds of a North American bienothere by G. Edward Lewis. Crompton and Ellenberger (1957) have made an important contribution in their description of the dentition of a cynodont of the Family Traversodontidae which shows strong resemblance for a cynodont-tritylodont relationship. The ictidosaurs, which appear to have arisen from scaloposaurid therocephalians, and the tritylodonts must be considered as separate groups, as these authors have suggested.

Two finds of Rhaetic or Liassic (uppermost Triassic or early Jurassic) mammals are most important additions to our knowledge of the reptilian-mammalian transition. One consists of materials from fissure fills of the British Isles, with *Oligokyphus.* These are being studied by K. A. Kermack and co-workers. A second suite comes from the LuFeng series, associated with a small species of *Bienotherium.* These are from the collections made available for study of Father Rigney, and are being studied by Bryan Patterson of Harvard University and me. It would appear that these two suites of early mammals represent respectively docodonts [1] and a different group with teeth remarkably like those of some of the "advanced" triconodonts. In each group there is some suggestion of the existence of more than a single element in the lower jaw. By every criterion, except that of the lower jaw, these new finds are mammalian. If we are to be rigid in application of the criterion of jaw structure, they probably would have had to be called reptiles, but if this is done, the idea of "reptile" must be stretched beyond all practical limits.[2] If this is not done, what becomes of the position of the bienotheres, or the ictidosaurs: mammals or reptiles? Really, of course, this situation approaches the ideal for it shows that

[1] Based on a preliminary statement by Dr. Kermack at the meeting of the Society of Vertebrate Paleontology, 1957 and now confirmed in a published statement, Kermack and Mussett (1958).

[2] Shortly after this paper had been presented in a symposium of the Society for the Study of Evolution and the Society of Vertebrate Paleontology, in very early September, 1958, at Ann Arbor, Michigan, the detailed account of triconodonts from the British Isles, by W. G. Kühne, published in August, 1958, became available (Kühne, 1958). In this report the author gives considerable attention to the sources and origin of mammals. Since, however, presentation of present paper preceded reception of Kühne's work, it has been considered best to treat it in a footnote rather than through modifications of the text.

In his report, Kühne proposes that the Pantotheria, Amphilestidae, and Triconodontidae stemmed from *Morganucodon.* He points out that these forms all have a persistent sulcus for Meckel's cartilage and presumably a double condyle of the lower jaw, formed by the dentary and articular. The source of *Morganucodon* he considers to be among the cynodonts. The "mammalian" forms, except Pantotheria, are considered transitional between therapsids and mammals and the origin of true mammals to have taken place within the Order Triconodonta. Symmetrodonts are thought to have arisen independently from cynodonts. Tritylodonts, on the other hand, have only one element, the articular, involved in the condyle.

Kühne's paper has a most important bearing upon the sources of mammals and, if followed, would modify the patterns shown in figure 1 of the present paper as well as a number of the conclusions of Patterson (1957). Also, the transition to mammals is shown as Liassic. In these ways, his report has an important bearing upon some parts of the present paper. Since, however, it does not bear directly upon the major theme, I feel it best to attempt no comment upon the implications other than to note that they must be given consideration in any study concerned with the origin of mammals and the early deployment within this Class.

we are gaining an insight into the actual transition.

Finally, somewhat more remote from our central theme, the lower Cretaceous mammals of the Trinity formation of Texas, as described and discussed from Patterson (1957), provide vital information upon the early courses of mammalian evolution. The distinctness of early mammalian lines is emphasized and, in particular, the isolated position of the docodonts among the mammals is significant.

These finds and studies bring us to a position more favorable for consideration of various aspects of the problems of evolution of the mammals from their reptilian predecessors. In this paper, I shall consider very briefly, as a basis for a following discussion, the patterns of evolution of the mammalian predecessors. I will then turn to what appears to me to be the heart of the matter, the independent development of mammal-like characters in the various evolving lines of therapsids.

Patterns of Evolution of Mammalian Predecessors

A phylogeny which represents the general patterns of evolution as they look to me at the present time is given in figure 1. While this is undoubtedly incorrect in many details and will certainly be subject to both major and minor revisions in the future, it does serve well as a pictorial basis for brief consideration of certain key events in pre-mammalian evolution. Five "critical" stages are indicated by the letters "A" through "E" on the diagram.

"A" represents an adaptive shift from a primitive captorhinomorph. On the one hand there is an herbivorous line and on the other, one which is carnivorous. Ophiacodont and sphenacodont pelycosaurs represent derivatives of the carnivorous line. Whether the herbivorous pelycosaurs, the edaphosaurids, stemmed from an originally carnivorous line among the primitive pelycosaurs or, in fact, represent a group derived independently from captorhinomorphs is uncertain at present. The assumption of carnivorous habit is, to me at least, a critical affair, for by this step, the stage is set for initiation of increasing activity, which is a primary feature of the trend toward the condition we call mammalian.

There followed an adaptive radiation of the pelycosaurs with the development of advanced ophiacodonts, sphenacodonts, caseids, and edaphosaurids. The latter two groups assumed herbivorous modes of life. Two important points with respect to this radiation require emphasis. First, we know of it only from faunas from very low-lying areas that appear to have lain close to the oceanic margins of the continents. It is primarily late Carboniferous and early Permian in time and, where it extends into the middle Permian, its elements are, for the most part, found only in persistent deltaic deposits. The second item of importance is that in the course of this radiation there was a great deal of adaptive divergence but little consistent tendency in the various lines to develop increasingly mammal-like characters. The sphenacodonts do become somewhat more mammal-like in the course of their evolution but even here we find but slight evidence of the phenomenon of independent acquisition of mammal-like features in the different lines.

The events marked as "B" represent the transition from sphenacodont pelycosaurs to therapsids. This is a gradual change and the placement of transitional genera in one group or the other is a matter controlled more by geography and stratigraphy than by morphology. Such elements are known both in Russia, in zones 1 and 2, and in North America in the San Angelo. Most details of the transition appear to be lost as a result of lack of suitable non-deltaic environments in the waning stages of the lower Permian.

The events marked "C" represent the earliest phases of therapsid radiation. As in the pelycosaur radiation, both carnivorous and herbivorous forms developed, for there was a strong and diverse adaptive

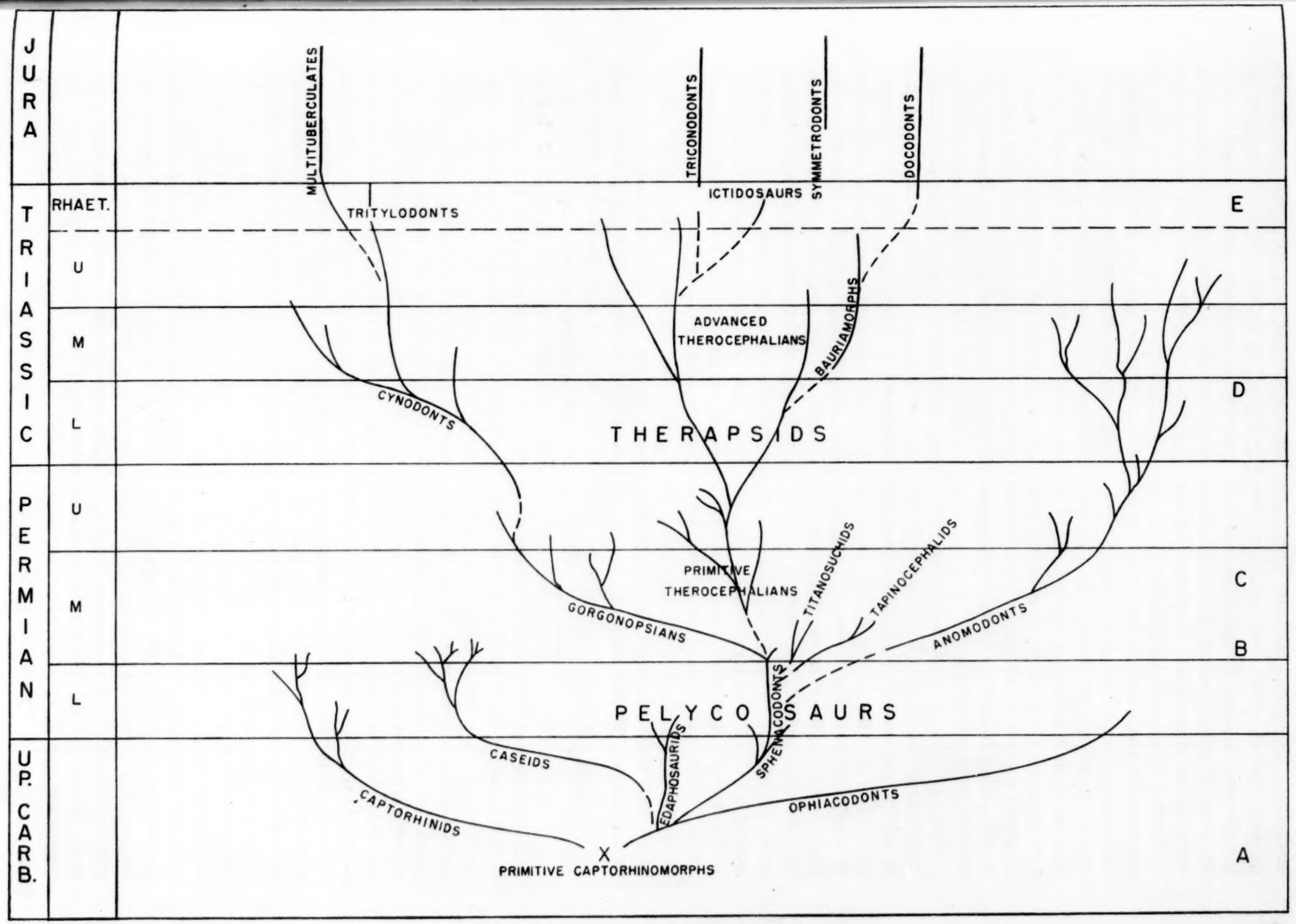

FIG. 1. Evolution of mammalian predecessors.

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evolution. In these various lines, the tapinocephalids, the titanosuchids, the gorgonopsians, the therocephalians, and the anomodonts, however, there appeared, independently, incipient features, which in their fruition and integration come to characterize the state we may call "mammalness." Stage "D" represents the advanced therapsid radiation. In the various lines, now widely separated, there is a continuing acquisition of mammal-like characters, and "perfection" of those mammal-like features derived from more primitive representatives.

Finally, "E" represents the transition from reptiles to mammals. This is shown as taking place in several lines along the pattern proposed by the writer in 1944 (Olson, 1944). The concept of multiple origin of mammals as more or less formalized in that report was not, of course, new. Since that date, it would appear, however, that the great majority of students of this segment of evolution, with a few notable exceptions, have considered the implications of this representation to be a fair approximation of the events that lead to mammals.

Interpretation of the Evolutionary Patterns

If the sequence of events suggested in figure 1 can be considered a proper presentation of the broad pattern of mammalian evolution, we have, I believe, made significant progress in arraying of the facts gleaned from the record. To me, at least, a most important aspect of our task of elucidation falls in the area of interpretation of the factors that have causal relationship to the patterns that are observed. The most striking feature of the history of the mammal-like reptiles is the independent acquisition of mammal-like characters by the various therapsid lines with the resultant continuing increase of mammalian habitus. It is upon this feature that I will focus attention in the rest of this paper.

In essence, this pattern of evolution represents a case of multiple directional evolution within a coherent taxonomic group at an ordinal level. Examples of directional evolution are plentiful in the fossil record. Cases of multiple lines at a familial or higher level, are less common and often difficult to recognize because of the confusion that similarities introduce into taxonomic determination. The amphibians and the holostean fish show evidence of this pattern, and there are probably a number of other valid cases. At present, however, the therapsid case is probably the most precisely documented.

To me, at least, this has been a challenging phenomenon that has posed a number of interesting problems. To set these more precisely, I will depart for the moment from direct consideration of interpretation to summarize what seems to be involved. First, the various evolving lines of therapsids existed in the same general environment at the same time. Second, each line had its own adaptive evolution. Third, there are, in the same beds and presumably in the same general environment, other reptilian groups which show both similar and different adaptive modifications. Fourth, there appear in each of the lines of therapsids, irrespective of their adaptation to one or another facet of the environment, common characters, which are usually more fully expressed sometime after their origin than when they first appear. To some extent, some of these characters show an impact of the adaptive modifications in a particular line, but this is the exception rather than the rule. Few of the characters and none of the suites of characters under consideration appear in other reptilian groups.

The various features show no evidence of sudden acquisition, but rather appear as minor modifications which gradually become better expressed. Briefly some of the most striking are as follows: in the skull and jaws we find initiation and development of the secondary palate; reduction in thickness of the basicranium; exclusion of the epipterygoid from the cranio-palatal joint; expansion of the epipterygoid to a mammalian alisphenoid

pattern; reduction of the relative size of the *sella turcita;* broadening of the basal part of the brain case; deepening of the subarcuate fossa to form a well defined and deep flocular fossa; rotation of the vertical semicircular canals, with the anterior becoming short and straight and the posterior long and strongly curved; loss of strong ossification in the *pila antotica* (pleurosphenoid bone) and in the *taenia marginalis;* and development of a double occipital condyle. Postcranial trends are less easily sorted out, but some may be noted; development of a mammal-like atlas-axis complex; reduction of coracoids and cleithrum; rotation of the originally vertical axis of the pelvis; and reduction of the phalangeal formula. It will be noted that the modifications of jaw structure have been omitted. These do proceed independently, but represent continuations of trends established in the sphenacodont pelycosaurs.

Some of these characters seem specifically adaptive. Most, however, are adaptive in a somewhat modified use of the term, in that they have "general" rather than "particular" adaptive significance. They are for the most part rather different from the strictly adaptive features found in lines as they diverge into various subzones of the environment and become better suited to exploitations of particular conditions.

Since phenomena of directional evolution are seen primarily in the fossil record the majority of speculations about them have been by paleontologists. Some have proposed one or another form of vitalistic causation, whereas others have approached the problems from the point of view of genetics and selection. There are interesting problems where but a single line is involved, but these are considerably compounded when we have multiple lines and where the trends flourish in the face of highly varied adaptive patterns.

Various explanations can be and have been proposed. One involves the idea that the complex genotype was strongly predisposed to certain changes and sequences of changes that are reflected through time by the phenotype. Or it can be thought that the characters expressed were latent in the common inherited genotype, and not expressed phenotypically until some time after divergence had occurred. The action of suppressive elements in the genotype may be invoked to augment this concept. Frequently, where there is no evident adaptive aspect of a character or where it seems actually deleterious, the concept of linkage to important features is cited. A special case is where a character as initiated has no apparent value but later assumes some important role. Probably all of these suggestions plus others have some element of truth in them, but none seems sufficient or entirely satisfactory to me in the present case.

Consideration of characters of the sort we have noted, their way of appearance and development, plus various similar considerations, have at times in my own mind and I am sure in the minds of others, given rise to an uneasiness with regard to the current genetic-selective concepts of evolution. This has been expressed in particular by some invertebrate paleontologists, who have either proposed contrary approaches, for example, Schindewolf (1954), or have merely abandoned efforts of interpretation and thus have left their opinions unvoiced in the literature. There has also, however, appeared an increasing, although specifically unexpressed, tendency of vertebrate paleontologists, especially in England, to "leave well enough alone" the problems of causation of evolution and to restrict their work to description of the record. In part this appears to stem from difficulties of reconciliation of results from recent materials and those of the fossil record.

Where then do we stand, especially with respect to the apparent dualism in the therapsids? Are we dealing with two kinds of systems, one for now and one for the future? Are we seeing in the fossil record something that does not come to light in laboratory and field

studies of evolution? Is there a gap in the current theory, which while it may be correct, is not sufficient? Or are we being deluded by a faulty record which gives false interpretation of the events we believe it records?

I believe that the answer to each of these questions is in the negative. Also I deplore the tendency toward abandonment of efforts to seek solutions, even though answers must remain tentative. Rather, I think that whatever fault there may be lies in part in the way that some of us at least, myself included, have tended to look at the evidence. I will not dwell upon the need of a population concept for I am sure that we all recognize it whether or not we use it in practice. It may merely be noted that I consider it essential to think about the therapsids in terms of temporally successive populations and population complexes modifying through the process of speciation. What I should like to discuss, however, is the predilection to look too restrictively at just what is provided for examination by the record, that is preoccupation with preserved characters, singly or in small numbers, *per se*.

A great many of the features that we observe in our fossil materials may best be considered as "symptomatic" of adaptation rather than, of themselves, primarily adaptively significant with respect to the external environment. They may point a way to understanding, but cannot in themselves best be considered solely or primarily in their relationship to external environment. Perhaps we do not think this way, but from what I read and hear, I cannot avoid the feeling that in general we do. Perhaps I can clarify my point by suggesting that it seems more profitable to think of many characters as basically adapted to the organism and secondarily, if at all, adapted to the external environment. Understand, I am not attempting to establish a rule that I believe must be followed for all characters. Rather, there may be conceived some characters that are about equal in their adaptive relationship to external and internal environmental situations, and, spread about such "mean" characters, a distribution that extends to near complete dependency in both directions. It is primarily the characters that emphasize relationship to internal features with which I am concerned and which, I feel, have posed the elements of confusion.

Viability, it seems safe to assume, is primarily related to physiological processes. Many ostensive characters of the phenotype, whose adaptive significance we may seek, whether or not they provide for a reasonable interpretation, probably have little or nothing to do do with the success of the particular organism in reaching reproductive maturity. To have selective advantage, they must operate to increase the probabilities of such viability or in some other way promote the chances of reproduction. Certainly some of the characters observable in fossils act in this way, but in many, or perhaps, most cases, it seems extremely risky to infer primary selective value merely on the basis of after the fact evidence of persistence or interpretation with respect to a single specific function. Not the least of the danger is the fixation of attention on the single item, which obscures the part that it may play in the total complex of the organism, which is the primary unit upon which selective action operates from the time of conception.

In the framework which recognizes physiological processes as dominant in the success of an organism, the patterns of evolution seen in the therapsids find ready explanation and it may well be that other cases can be clarified as well. In fact, the solution of the problems of aquisition of suites of similar characters in a series of distinct but related multiple lines yields so readily to such an interpretation that I cannot but feel that others will say, "Of course, that's what I have been saying all along." Somehow, if this is true, I have missed the boat, for until recently this problem has been very puzzling to me.

As has frequently been stated, the key to evolution of the mammals, relates to increasing activity. But, of course, other groups of reptiles also became very active and showed no approaches toward what we call "mammalness." From one such line came the birds. We know that a result of therapsid-evolution was homoeothermy. So was a result in the evolution of birds, but the structural modifications were in most respects very different. There is some evidence that there were "experiments" with blood temperature control in some of the pelycosaurs, e.g. in the long spines of such forms as *Dimetrodon*. It appears that the therapsids arose from the most active of the known pelycosaurs, the sphenacodonts. It is evident that in the pelycosaur evolution, there are very few cases of independent aquisition of similar characters in different lines and none of acquistion of similar suites of characters. Only with the development of the therapsids did this take place. Thus there appears to have developed, late in sphenacodont history, a threshold which cast subsequent evolution into a new framework. I suggest, from the remarks on homoeothermy above, and from the nature of many of the independently acquired characters, that this threshold represents the inception of a basic physiological change, and that this change is the development of incipient homoeothermy.

Several interacting phenomena are called into play in a rational explanation. The genotype inherited by all therapsids had a common building plan and presumably this organization was to some extent expressed in the physiology and soft anatomy of the late sphenacodonts. Clearly, within the physical environments into which most therapsids radiated, uplands away from deltaic lands, there would be a strong selective value in increase of homoeothermy, in large part irrespective of the particular ecological zone or subzone which a particular stock came to occupy. Any change or series of changes in structural components that were advantageous to the perfection of homoeothermy, whether in relationship to external environment, internal environment, or both, would promote the selective advantage of the organism. It is this sort of character, as we see it in the preserved individual, that I feel should be looked at in terms of its adaptation to this common and vital physiological process.

In the therapsids, as in any taxonomic category, the genotype operating through the stages leading to adulthood imposed limitations upon the nature and degree of changes permissible for viability. Thus only limited types of changes were possible. With continuing selection favoring the physiological process of homoeothermy any feature which appeared and was favorable to this process would tend to have selective advantage. In view of the limitations imposed by heredity, the nature of the variability produced by the genotypic modifications would tend to be very similar in recently diverged populations and selection related to homoeothermy would tend to produce very similar results. These results, in turn, would themselves become restrictive and insure limitations of the possible selective avenues in successive populations.

This holds, of course, only if we consider the characters in question as primarily adapted to some underlying, continuously selected feature of the organism. Adaptation to particular external conditions, for example to a particular type of food, to mating patterns, to locomotion, could proceed through exploitation of variations in the same genotype in any direction for which variation provided the raw materials. Probably no changes can be considered totally independent, but the degree of interdependency clearly varies greatly. To the extent that adaptive changes primarily related to external environment were compatible with the changes concerned most intimately with physiological change, both the process of general improvement, that is increased effectiveness in many types of environment, and process of special adaptation

could proceed together in harmony. If the latter attained ascendency, that is if the immediate adaptive advantage outweighed the advantage of physiological improvement, we should expect to see cessation of the aquisition of characters adapted to the physiological process. This is what may have happened in some of the anomodont lines where this cessation and even some retrogradation of some of the suites of characters occurs.

Through this type of approach a rational and basically simple explanation of the phenomenon with which we have been concerned is at hand. On analysis, a great many of the characters which appear in varying temporal order, but apparently inevitably, in the therapsid lines can be logically related to homoeothermy. I think that the evidence of Brink and also his speculations point this rather clearly, although he is working from a different point of view. The relationships in some instances appear quite direct, such as those of the secondary palate and related masticatory functions. Others, such as modifications of neural structure inferred from the cranial changes are in part direct, but in part, for example the increase in the floccular area, less direct and related to body functions permissive and enhanced by increasing temperature stability. A detailed analysis of the various characters noted earlier in this light is hardly commensurate with the objectives of this paper, but even without explicit statement the way in which they fit logically into this general framework should be clear.

The Source of Mammals

If this general approach is followed, with recognition that physiological factors are the primary ones under selection, the concept of multiple origins of mammals is established on a firmer basis. Selection in all lines of therapsids will tend toward the mammalian condition and in any line in which there is full attainment of the physiological characteristics of the primitive mammals, we may expect expression of the secondary characters at essentially a mammalian level. In cases in which characteristics not primarily related to the developing physiology take ascendency in the selective hierarchy there will not be fruition of the total suite of ostensive characters of the skeleton which we call mammalian. Such lines will not then reach the mammalian threshold. We can say nothing about the development of reproductive mechanisms from any direct evidence except that cited by Brink, which seems very tenuous. However, glandular development in the skin which clearly has a selective value to developing homoeothermy, could pave the way for development of mammary glands. This in turn could set the stage for development of live birth at an immature level of development. The various stages of viviparous reproduction observed in mammals could have developed through relationship to the chain of events inferred in the evolutionary sequences which have been outlined.

What is a mammal and what is not a mammal, in the transition witnessed in the Rhaetic or Liassic, is pretty much an academic question and an *answer to it* depends in large part upon the objectives and philosophy of the one who poses the question. It seems to me that a satisfactory answer lies basically in the taxonomic convenience which it provides. If some genus ties in most logically with developing lines which are generally recognized as mammalian, it should be placed with the mammals. If it ties in more readily with some group of reptiles, which appears to have lead up to it, it should be placed with the reptiles. If we are ever blessed with such complete knowledge of the transition that some genus ties in equally well with a reptilian line and mammalian line, I presume that the best answer, if one must be had, is to be found in a flip of a coin. An alternative lies in a drastic modification of class structure as now commonly used and recognized. I believe that this is generally undesirable at present and that it is of

dubious value for the foreseeable future, since at best it can result in only a slightly more functional reconciliation of incommensurates, the actual evolutionary development and a valuable but necessarily artificial arrangement based on the concept of discrete sets.

Literature Cited

Brink, A. A. 1956. Speculations on some advanced mammalian characteristics in the higher mammal-like reptiles. Palaeontologia Africana, **VI**: 77–95.

Crompton, A. W. 1958. The cranial morphology of a new genus and species of Ictidosaurian. Proc. Zool. Soc. London., **130**, pt. 2: 183–216.

——, and E. Ellenberger. 1957. On a new cynodont from the molteno beds and the origin of tritylodonts. Ann. S. Afr. Museum, **44**: 1–14.

Efremov, I. A. 1950. Taphonomy and the geological record (in Russian). Trd. Paleon. Inst. Acad. Sci. U.S.S.R., **24**: 1–176.

——. 1954. Fauna of terrestrial vertebrates in the Permian copper sandstones of the Western Pre-Urals (in Russian). Trd. Paleon. Inst. Acad. Sci. U.S.S.R., **54**: 1–416.

——, and B. P. Vjuschkov. 1955. Catalogue of localities of Permian and Triassic vertebrates of the territories of the U.S.S.R. (in Russian). Trd. Paleon. Inst. Acad. Sci. U.S.S.R., **46**: 1–185.

Kermack, K. A., and Mussett, F. 1958. The jaw articulation in Mesozoic mammals. XVth Intern. Congr. Zool., Sect. V, paper 8, pp. 1–2.

Kühne, W. G. 1956. The liassic therapsid *Oliogokypus*. British Mus. (Natural History): 1–149.

——. 1958. Rhaetische Triconodonten aus Glamorgan, ihre Stellung wischen den Klassen Reptilia und Mammalia und ihr Bedeutung für de Reichart'sche Theorie. Palaon. Zeit., **32**: 197–235.

Olson, E. C. 1944. Origin of mammals based upon the cranial morphology of therapsid sub-orders. Spec. Paper Geol. Soc. Amer., **55**: 1–136.

——, and J. R. Beerbower. 1953. San Angelo formation, Permian of Texas, and its vertebrates. Jour. Geol., **61**: 389–423.

Patterson, B. 1956. Early Cretaceous mammals and the evolution of mammalian molar teeth. Fieldiana: Geology, **13**: 3–101.

Schindewolf, O. H. 1954. Evolution im Lichte der Paläontologie. Congres Geologique International. Comptes Rendus de la Dix-Neuvieme Session, U.P.I., XIX, 93–107.

EOCENE RADIATION AND PHYLOGENY OF THE RODENTS

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INTRODUCTION

The problems of the classification and evolution of the rodents continue to provide a basis for disagreement among students of the group. This is because of the considerable number of forms involved, and because of the unquestioned parallelism which has occurred in the evolution of members of the order. Even those authors who question parallelism in other structures admit that it occurs in the tooth patterns, and the present author believes it has been characteristic of *all* aspects of rodent morphology. There are many current problems which will be elucidated as more work is done, both on fossil and on Recent rodents. Until a great deal more of such work is carried out, the classification of the order cannot be anything but tentative. Nevertheless, such tentative classifications serve a value as showing areas where further investigations are needed, and in stimulating discussion, and the present paper is presented with this in mind.

For many years, most workers grouped the rodents into the three Brandtian suborders—Sciuromorpha, Myomorpha and Hystricomorpha (cf. Simpson, 1945) or some variant thereof (Tullberg, 1899). In the past few years, however, almost everyone working primarily with rodent taxonomy has expressed his firm conviction that these three suborders are not valid phyletic entities (see, for example, Lavocat, 1951, 1955, 1956; Schaub, 1953, a and b; Viret, 1955; and Wood, 1954, 1955). But almost everyone who has approached this question has come up with a different solution. There is only one way that the problem will ultimately be solved, and that is by the discovery and description of a series of fossil forms adequate to permit the phylogeny of all lines to be traced in detail, and to show how and when one major group has given rise to another. In the meantime, evidence from any point of view—morphologic, genetic, cytologic, serologic, embryologic, parasitologic, or other—that bears on rodent relationships will be of the greatest assistance. The present paper is an attempt to make a progress report on the matter, by giving a picture of the major branchings within the order, which seems reasonable to me on the basis of current knowledge, particularly of Eocene rodents and their history.

EOCENE RODENT HISTORY

The earliest known rodents, whether from North America or Europe, all belong to the family Paramyidae.[2] These forms appear in the later Paleocene of North America and the earliest Eocene of Europe. When they first appear they are rare, and represented by very fragmentary material. There is soon a greater diversity of rodents, so that two other families have already appeared, one in Europe and one in North America, before the top of the lower Eocene. In both cases, the limited evidence strongly suggests, though it does not prove, that the newly appearing families—the Pseudosciuridae in Europe and the Sciuravidae

[1] This study was assisted by grants from the National Science Foundation and from the Marsh Fund of the National Academy of Sciences.

[2] The earliest undoubted rodent material from the rest of the world is Oligocene. The only possible exception is the skeletal material described by Bohlin (1951, p. 18–27), probably from the Paleocene of Mongolia, which cannot be certainly identified, but which seems most probably to be a Lagomorph.

in North America—were derived essentially in place from the Paramyidae.

The sudden appearance of non-paramyids, particularly in Europe, has led some authors to postulate that the rodents had had a long previous history, inhabiting some areas of the world where they have not yet been found, and that the earliest paramyids are *not* ancestral rodents, but merely persistent survivors of the ancestral stock, which had originated perhaps as early as the Cretaceous, and which had undergone an extensive Paleocene radiation (Schaub, 1953a, p. 393; Lavocat, 1956, p. 55). Landry (1957, pp. 90–91) has reached similar conclusions though for different reasons. This, of course, is a postulate that it is impossible to prove or disprove at the present time, and will remain in this situation either until earlier non-paramyid rodents are found, or until a phylogenetic sequence has been established, showing the time of origin of the rodents. The choice of alternatives, therefore, is largely a matter of personal preference, depending on the individual student's previous bias. As Jepsen (1949, pp. 488–489) has pointed out, rodents are found, in the Paleocence, almost exclusively in a locality where the equally gnawing multituberculates are not found, which he interprets to mean that the rodents were then of recent origin, were expanding explosively from some unknown center of dispersal, and were rapidly replacing the multituberculates at the close of Paleocene time—a replacement which they completed during the early Eocene, and which opened up many ecologic niches to them. This hypothesis would explain our present knowledge of Paleocene rodents; the apparently very rapid diversification of the rodents in earlier Eocene; the quite primitive and fundamentally nearly tribosphenic appearance of the check teeth in the earliest rodents; and the rather striking changes in incisor pattern that took place among Paleocene and early Eocene paramyids. If Jepsen's suggestion is correct, it means very rapid evolutionary rates in Paleocene rodents, which would, of course, be expectable as they were busily invading new ecologic niches previously unavailable to them. This suggestion is explicitly supported by Wilson (1951, pp. 212–213). However long there had been rodents before the beginning of the Eocene, it seems probable, to me, that they had all been paramyids or pre-paramyids, and that the early Eocene members of the family give a clear picture of the original stock of the order.

After the appearance of the rodents in Europe and North America, a somewhat different evolutionary path was followed in the two continents. In Europe, the pseudosciurids gave rise in the mid-Eocene to the theridomyids, who continued with considerable diversity into the Oligocene. The paramyids are scarce in Europe throughout the Eocene, the Theridomyidae being the dominant middle and late Eocene rodents of that continent (Lavocat, 1951).

In North America, the paramyids remained abundant throughout the Eocene and into the Oligocene. At the same time, the Sciuravidae also diversified and became abundant. From some uncertain source but surely paramyid or sciuravid, a number of other lines developed, giving rise to several other families in the late Eocene.

Relationships of Eocene Rodents

What are the superfamilial relationships of the Paleocene and Eocene rodents? The criteria that have most generally been used for superfamilial arranging of the rodents are: the structure of the zygoma and infraorbital foramen and of the associated masseter muscle; the position of origin of the angle of the jaw; and the number and pattern of the cheek teeth.

Basically, the living rodents show three zygomasseteric patterns, related to the enlargement and development of an increased anteroposterior component by the masseter (fig. 1; B, C and D). In one,

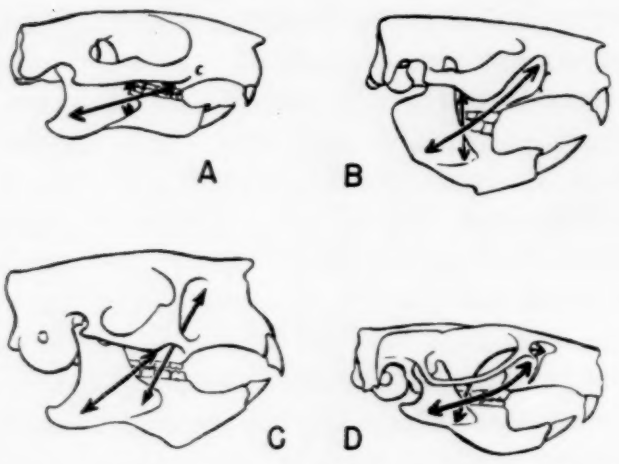

FIG. 1. Diagram of the four zygomasseteric patterns in rodents, showing paths of lateral and medial portions of masseter muscle. A. protrogomorph type. B. sciuromorphous type. C. hystricomorphous type. D. myomorphous type (modified after Romer, 1945, fig. 371).

the sciuromorph pattern, a branch of the masseter muscle has grown up in front of the orbit onto the side of the snout. In the hystricomorph pattern, another branch of the masseter has grown up, inside the zygoma, and has extended forward, through the greatly enlarged infraorbital foramen, again expanding onto the side of the snout. The myomorph pattern combines the other two, with both branches of the muscle expanding forward.

The angle of the lower jaw, arising from the posterior end of the mandible, very generally lies in the same vertical plane as the alveolus of the incisor. This Tullberg (1899) called the sciurognath pattern. In other rodents, however, it arises from the side of the jaw, lateral to the incisive alveolus—his hystricognath pattern.

The cheek teeth of rodents were originally derived from the tribosphenic pattern. In a great many forms, transverse crests have developed in these teeth, consisting of ridges between the four main cusps of the upper and lower teeth, plus anterior and posterior cingula, and, in many forms, plus a fifth transverse crest that developed in the center of the teeth. Hypsodonty or hypselodonty has appeared at any stage in this development, and there may be marked similarities between ultimate tooth patterns of different lines, without there being any necessary relationship.

Many other criteria have been used for dividing rodents into major groups, with greater or lesser success. Characteristics of the soft anatomy, such as those of the male reproductive tract, have been proposed by some authors (Pocock, 1922; Mossman, Lawlah and Bradley, 1932) as being of fundamental significance, but they obviously cannot be used in dealing with fossils. The structure of the ear ossicles, recently stressed by Landry (1957, p. 16), is also of very dubious value in working with fossils due to the almost complete absence of specimens preserving these bones. Unfortunately, no adequate comparative studies (to include individual variability) seem to have been made on these structures in Recent rodents. The baculum also is essentially unknown in fossil rodents.

The attempts to select one or another of these structural developments as *the* critical one have not led to a solution of the problems of rodent classification, and many of those who have studied rodent evolution have been impressed with the certainty that each of the adaptations of these structures has developed more than once, independently in different lines of rodents.

The three basic criteria (zygomasseteric pattern, angle, and cheek tooth pattern), however, are still the best structural features available for studying rodent phylogeny, as long as they are treated as a guide to rodent relationships and not as keys to rodent classification. For example, using them as a group, it becomes clear that the Theridomyidae stand apart from the other Eocene rodents. This family has developed the hystricomorphous infraorbital foramen and jaw muscles and has acquired 5-crested hypsodont or hypselodont cheek teeth, while retaining the primitive sciurognath condition of the angle. The Pseudosciuridae are clearly ancestral to the theridomyids, and seem surely to have been, in turn, derived from the Paramyidae. The Theridomyidae

were formerly universally accepted as a basal group of the hystricomorphs, and are still considered by many workers, especially in Europe, to occupy such a position, though this does not seem reasonable to most American workers.

One other family, the late Eocene and later Eomyidae, also stands apart in zygomasseteric structure, having attained the sciuromorphous condition, although it is reasonably close to the Sciuravidae in dentition and in such other features as are adequately known, including the sciurognath angular structure. The remaining Eocene rodents (with the exception of one or two forms of dubious relationships) are all obviously closely related. They are all, however, distinct from the three classic suborders (Sciuromorpha, Myomorpha and Hystricomorpha), and deserve recognition as a fourth suborder, the Protrogomorpha, characterized by: the masseter muscle unmodified, its origin limited to the zygoma in the primitive mammalian fashion (fig. 1A); the infraorbital foramen generally small; usually (though perhaps with some exceptions) sciurognath; and cheek teeth cuspate or lophate, high crowned or low crowned, but never based on a five-crested pattern. This group also includes the mid-Tertiary Mylagaulidae and Aplodontidae, including the single living genus *Aplodontia*.

Phylogeny of Later Rodents

If most of the known Eocene rodents belong to the Protrogomorpha, what conclusions can be reached as to the validity and time of origin of the remaining subordinal groups of rodents?

The difficulty in assigning all Recent rodents to the three classic suborders is emphasized by the fact that there are always a few left-over groups, which do not fit well into any classification that has been proposed. Unfortunately, most of these problematic groups are also very poorly known as fossils, which does not help in understanding their relationships. It seems best, therefore, to attack the problem of subordinal relationships by concentrating on those groups of families where there is evidence of relationship, and where there is a fairly good paleontologic record, to see what pattern can be determined.

One of the largest groups of rodents which are generally agreed to be related is the group of South American families which have been considered to be hystricomorphs, and which Wood and Patterson (Wood 1955, p. 180; Wood and Patterson, 1959) have called the Caviomorpha. There seems to be general agreement that this group invaded South America, presumably from North America, and carried out its radiation in South America, there very probably having been but a single invading stock. But considerable differences of opinion remain as to what this stock was, and when the invasion occurred. In the structure of the infraorbital foramen, the caviomorpha are hystricomorphous. It should be noted, however, that in *Platypittamys*, one of the earliest genera and one represented by essentially complete skeletal material, the infraorbital foramen is unusually small and there is no evidence that it was traversed by the masseter muscle, which are conditions that would be expected in a precaviomorph condition (Wood, 1949, p. 13; Wood and Patterson, 1959, p. 299). In the structure of the angle of the jaw, the caviomorphs are hystricognath, as in the hystricomophs and various other rodents, including, incipiently, some paramyids. The most primitive caviomorphs, from the lower Oligocene Deseado Formation, show a cheek-tooth pattern that is basically 4-crested, whereas the Old World forms that have been referred to the Hystricomorpha are apparently all 5-crested, which seems a fairly significant difference. No South American rodents are known before the Deseado. This means either that they did not exist in South America during the Eocene, or that they have not yet been found. In view of the relative

closeness of the families and genera of Deseadan rodents to each other, it seems certain that their common ancestor had lived not long before, and that this ancestor had only very recently invaded the continent, from North America. While the phylogenetic sequence is a long way from complete, there are North American Eocene paramyids which, as far as their morphology is known, *could* have given rise to the Caviomorpha, and there are no known non-protrogomorph rodents from any part of the world that could have done so. The simplest interpretation would therefore seem to be that the Caviomorpha are a natural group, and that their structural similarities (of which there are many) to any other Oligocene and later rodents are due to parallelism.

Recently Wilson (1949, p. 113), followed by Wood (1955, pp. 176–179), has suggested that a variety of rodents of mouse or rat size (including the typical myomorphs such as the Muridae and Cricetidae; the atypical sciuromorph group of the Geomyidae and Heteromyidae and their probable relatives, the Eomyidae; and the atypical myomorph group of the Dipodidae and Zapodidae) are all distantly related to each other, in spite of differences in their masseter muscles, because of basic and apparently rather fundamental similarities, particularly in cranial structure. These families are here considered to represent the basis of the revised suborder Myomorpha, to which the Spalacidae, Rhizomyidae, Gliridae and Selevenidae have rather hesitantly been added. These families seem to have had a common ancestry, probably about mid-Eocene, which may well have been protrogomorph in masseter structure and very possibly taxonomically a sciuravid. Specializations of the jaw muscle structure would then have developed within this sciuravid group, so that probably at least three separate lines crossed the technical boundary between the Protrogomorpha and the Myomorpha—the Muroidea and the Dipodoidea, which became more or less myomorphous, and the Geomyoidea, which became sciuromorphous. The Rhizomyoidea and Gliroidea may represent two other such lines. That rather diverse patterns of masseter muscle development can occur in related forms is shown in the protrogomorph family Ischyromyidae, where the genus *Titanotheriomys* show a sub-sciuromorph structure (Wood, 1937, p. 195), while the closely related genus *Ischyromys* does not (although the type skull of *I. troxelli* and one specimen of *I. pliacus* in the Amherst collections seem also to be developing in the sciuromorph direction).

After the removal of the Protrogomorpha and the Geomyoidea from the classic Sciuromorpha, this group is reduced almost to nothing.

There are many difficulties in grouping the squirrels and beavers together, particularly exemplified by the striking differences in their teeth, and they have recently been separated (Wood, 1955). The Sciuridae can be followed back, without much question, into some of the central members of the Paramyidae. Unfortunately, these latter are both rare and fragmentary, so that this sequence is based largely on the cheek tooth pattern. But no other known group could have given rise to the Oligocene and later squirrels. The beavers, when followed back to their earliest appearance, show no signs of tying in with any other families except the Eutypomyidae.

All the other families of rodents can be traced for longer or shorter distances in the geologic record, but they do not tie together except in small groupings. The relationships of most of these must be considered as remaining problems for future work. An attempt has been made to indicate on the phylogenetic tree (fig. 2) what seem to me to be reasonable relationships. Undoubtedly many are incorrect, but progress cannot come from continuing to unite forms for whose relationship there does not seem to be adequate basis.

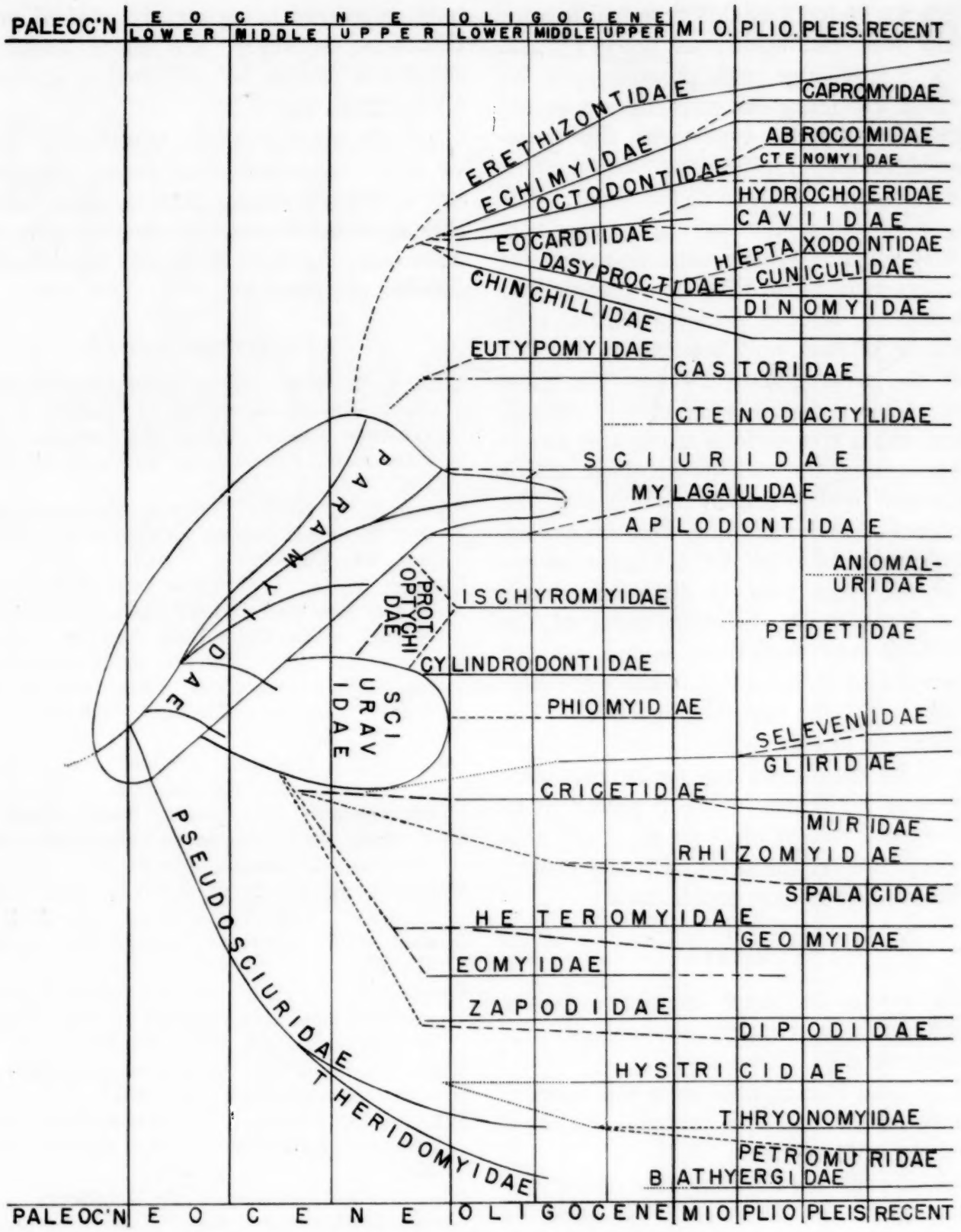

FIG. 2. Suggested phylogeny of the families of rodents.

RODENT RADIATIONS

The initial rodent radiation of the Eocene was based on a protrogomorph condition, which fundamentally meant chewing with the temporalis muscle, giving a vertical movement to the lower jaw. There was an anteroposterior component, brought about largely by the masseter but in part by the anterior part of the temporalis, which was used in gnawing. This, however, was relatively inefficient.

Beginning in the middle or later part of the Eocene, there was a much larger rodent radiation, in which the emphasis was shifted to the masseter muscle, as this muscle spread forward onto the face.

Since the rodents by this time had become fairly well established as gnawers, and since this change was providing an increased efficiency in gnawing, it is not surprising that the types with this structure developed rapidly and soon became the dominant members of the order. The selection was not, however, for any particular zygomasseteric condition, but for changes which permitted the masseter to develop a strong anteroposterior pull. Because of this, and because of the fact that the protrogomorph forms that gave rise to this radiation were closely related, there was a tremendous amount of parallelism among the rodents involved in this radiation, which greatly complicates the problem of understanding what happened. While this radiation did not occur at exactly the same time in different groups of rodents or in different parts of the world, it was essentially ended (as far as the major structural patterns were concerned) by the early Oligocene. The elucidation of this radiation, and the tracing of its details are the major problems currently needing solution to permit us to understand rodent phylogeny. Until this is done, the relationships of many rodent families must remain problematic.

Summary

In conclusion, there are a few points which seem to be very probable, if not demonstrated. These are:

(1) The Paramyidae were the ancestal family of rodents, and probably included all Paleocene rodents;

(2) From the Paramyidae there developed, in the Eocene, a radiation of Protrogomorpha, forms in which the masseter remained primitive;

(3) The middle to late Eocene radiation gave rise to the sciuromorphous, myomorphous and hystricomorphous types of rodents, each of which seems certainly to have developed independently more than once;

(4) The superfamilial grouping of the order is complex, and the details are a long way from being clearly worked out. Certainly, however, the three classical suborders cannot be stretched to include all rodents; and

(5) In view of all the parallelism that we know has taken place during the evolution of the rodents, the only way that a firm classification can be developed is by understanding the phylogeny of all the families involved.

Literature Cited

Bohlin, B. 1951. Some mammalian remains from Shih-ehr-ma-ch'eng, Hui-hui-p'u area, Western Kansu. Rept. Sino-Swedish Sci. Exp. N.W. Provinces of China, Publ. **35**: 1–47.

Jepsen, G. L. 1949. Selection, "Orthogenesis" and the fossil record. Proc. Amer. Phil. Soc., **93**: 479–500.

Landry, S. O. 1957. The interrelationships of the New and Old World hystricomorph rodents. Univ. Calif. Publ. Zool., **56**: 1–118.

Lavocat, R. 1951. Révision de la faune des mammifères Oligocènes d'Auvergne et du Velay. Paris: Editions "Sciences et Avenir": 1–153.

——. 1955. Quelques progrès récents dans la connaissance des rongeurs. Colloque internationale d'Evolution. Paris: 77–85.

——. 1956. Réflexions sur la classification des rongeurs. Mammalia, **20**: 49–56.

Mossman, H. W., J. W. Lawlah and J. A. Bradley. 1932. The male reproductive tract of the Sciuridae. Amer. Jour. Anat., **51**: 89–155.

Pocock, R. I. 1922. On the external characters of some hystricomorph rodents. Proc. Zool. Soc. London, **1922**: 365–427.

Romer, A. S. 1945. Vertebrate paleontology. Univ. Chicago Press. ix + 687.

Schaub, S. 1953a. La trigonodontie des rongeurs simplicidentés. Ann. Paleont., **39**: 29–57.

——. 1953b. Remarks on the distribution and classification of the "Hystricomorpha." Verh. Naturf. Ges. Basel, **64**: 389–400.

Simpson, G. G. 1945. The principles of classification and a classification of mammals. Bull. Amer. Mus. Nat. Hist., **85**: i–xvi, 1–350.

Tullberg, T. 1899. Ueber das System der Nagethiere: eine phylogenetische Studie. Nov. Act. Reg. Soc. Scient. Upsal., (3), **18**: 1–514.

Viret, J. 1955. Rodentia fossiles. La denture des rongeurs actuels et fossiles. Traité de Zoologie. Anatomie, Systématique, Biologie,

edited by P.-P. Grassé. Masson et Cie., Paris, **17**, pt. 2: 1526–1564.

Wilson, R. W. 1949. Early Tertiary rodents of North America. Carn. Inst. Wash. Publ., **584**: 67–164.

——. 1951. Evolution of the early Tertiary rodents. Evolution, **5**: 207–215.

Wood, A. E. 1937. Rodentia. Part II in The mammalian fauna of the White River Oligocene, by W. B. Scott and G. L. Jepsen. Trans. Amer. Phil. Soc., n.s., **28**: 157–269.

——. 1949. A new Oligocene rodent genus from Patagonia. Amer. Mus. Novitates, no. 1435: 1–54.

——. 1954. Comments on the classification of rodents. Breviora, **41**: 1–9.

——. 1955. A revised classification of the rodents. Jour. Mammalogy, **36**: 165–187.

Wood, A. E., and B. Patterson. 1959. The rodents of the Deseadan Oligocene of Patagonia and the beginnings of South American rodent evolution. Bull. Mus. Comp. Zool., **120**: 279–428.

UNIVERSITY

ROUND TRIP FROM SPACE[1]

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Whence came life on the surface of the earth? Whether or not a complete answer to this question may be found within the context, and content, of modern science remains a moot question. It is our purpose to see how far we can devise an answer, and how satisfactory it may be, within that context. It becomes clear immediately that we will be dealing not only with biology but all of the contiguous sciences—physics, chemistry, geology, astronomy and the like. However, our primary point of view will be that of biology and chemistry. At every point of our discussion, we will limit ourselves to asking questions, and proving answers, which, at some point, may be susceptible of observational, or experimental, test.

WHAT IS LIFE?

Since we have phrased the question in terms of "the origin of life," we presumably have a clear conception of what we mean by the term "life." There is very little doubt but what, on the ordinary level of human experience, there is no difficulty in distinguishing that which lives from that which does not. However, when we explore this notion to try and determine precisely what it is, or to be even more specific, what qualities we must devise, in order to produce something which lives from something which does not with no help from a living agency save the hand of a man, the question becomes somewhat more ambiguous. For example, there are many qualities which we have no difficulty in attributing to a living organism. It is able to reproduce itself, to respond to an environmental change, to transform energy into order (sunshine into a leaf, leaf into a hair), to change and remember the change, etc. While many of these individual characteristics may be duplicated in systems in space, one or more at a time, it is only when a sufficiently large collection of them appears in a single system in space that we call that system alive.

Thus, the definition of life takes on the arbitrariness of the definition of any particular point on a varying continuum, and precisely where that point will fall on the line of time and evolution will depend a good deal upon who is watching the unfolding of that line.

With this last remark, we have introduced the basic notion of evolution which, since its precise formulation exactly one hundred years ago by Darwin and Wallace, has pervaded all of science. In fact, most of what I have to say could be formulated in terms of a long extrapolation backward in time of the notions that were so ably expressed by Darwin and Wallace in 1858, but which they did not extend very far back, either in geologic time or certainly in cosmic time (Darwin and Wallace, 1858).

For a period of over sixty years any serious discussion of the question of the origin of life was not indulged by scientists, particularly by experimental scientists. In fact, it was considered a disreputable kind of activity. It is interesting to examine what some of the reasons for this might have been. One certainly (and perhaps a dominant reason) was the dictum of a contemporary of Darwin's (1809–1882), a chemist, Louis Pasteur (1822–1895), who, in 1864, quite clearly provided an answer to the question which,

[1] The preparation of this paper was sponsored by the U. S. Atomic Energy Commission.

for many decades previous, had been the subject of much discussion, namely, the possibility of the spontaneous generation of life on the earth today. Pasteur quite clearly, and definitely, established, in his experiments of 1864, that it was impossible on the earth today, under controlled conditions, to demonstrate the appearance of living material except through the agency, or as offspring of, other living material. While Pasteur opposed the Darwinian formulation of evolution, largely on religious grounds, I suspect that he either knew consciously, or felt instinctively, that the Darwinian doctrine was conceptually in conflict with his experimental conclusion (Sears, 1950). A search of the works of Darwin has revealed no mention of his opinion of the conclusions that Pasteur reached in 1864.

In any case, for over sixty years thereafter, as mentioned earlier, there appeared practically no serious discussion of the origin of life, or spontaneous generation. Between the publication in 1870 by Alexander Winchell, a professor of geology, zoology and botany at the University of Michigan, of a book entitled, "Sketches of Creation" (Winchell, 1870) and the statements by J. B. S. Haldane, biochemist and geneticist, in 1928 (Haldane, 1928, see 1954) there appears to be no serious attempt to answer the question of the origin of life within the context of the science of the period.

The hiatus came to an end with the recognition by Professor Haldane that the dictum of Pasteur was not in conflict with the backward extrapolation of the doctrine of evolution as expounded by Darwin and Wallace, if one recognizes that at the time that spontaneous generation must have occurred, according to the evolutionary extrapolation of Darwin and Wallace, there was not, by definition, any living thing on the surface of the earth. Therefore, it was possible, in the prebiotic time, to accumulate large amounts of organic material generated by nonbiological processes. This, of course, cannot take place on the surface of the earth today, since there exist everywhere on the earth's surface organisms, both micro and macro, which would transform any such organic material immediately it is formed, even in small amounts. Thus, the apparent conflict of concept between the backward extrapolation of evolution and the dictum of "no life save from life" can be, and has been resolved (Oparin, 1957; Pauling and Itano, 1957). Since that time, that is, 1928, it has become increasingly popular amongst scientists (experimental and otherwise) to examine the question of the origin of life from the scientific point of view. In fact, it has become so popular that within the last 18 months there have been held at least two conferences in the United States and one international conference on the subject.

The Time Scale

It seems wise to have a look at the time we have in which to accomplish this total evolution of life. Here, in the first of our figures (fig. 1) something of the order of magnitude of the time with which we have to deal related to the geologic history of the earth can be seen. The earth was formed from matter in space some four to seven billion years

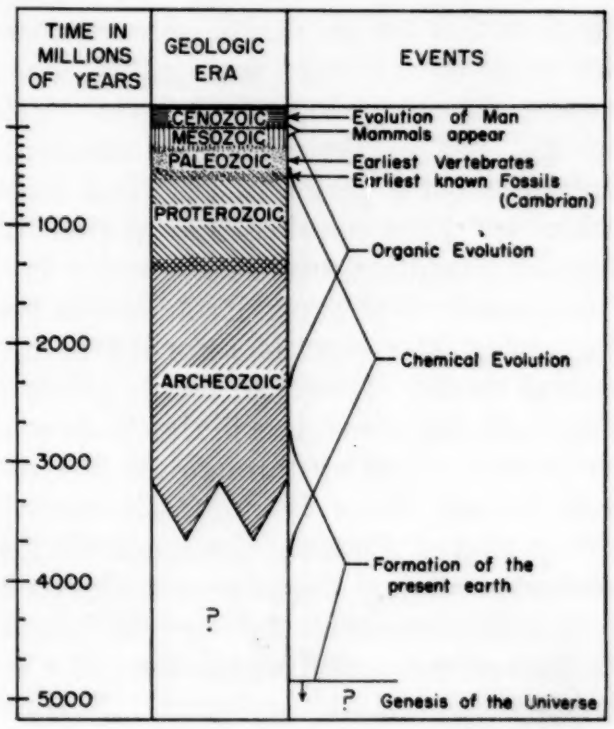

FIG. 1. The time scale for total evolution.

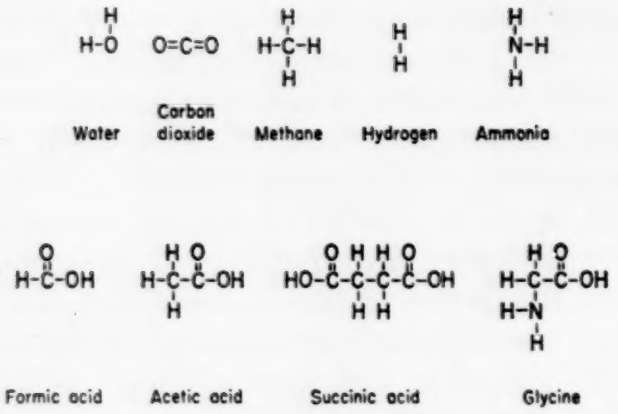

FIG. 2. Primeval and primitive organic molecules.

ago. Whether this was an aggregation of cosmic dust or a primeval explosion remains a matter of some controversy and will come up again a bit later in our discussion. However, that terrestrial history itself is only some five billion years in extent seems to be well established. It may be seen in figure 1 that the evolutionary periods on the surface of the earth are marked to correspond with the known geologic eras.

The earliest period might be spoken of as the period of the evolution of the present earth. Overlapping this, and including the Archezoic and Proterozoic geologic eras (some four to two billion years ago), is the principal period of which we will speak, namely, the period of Chemical Evolution. It was during this time that the formation of more complex organic molecules from simple ones occurred by nonbiological methods which we will try to describe in a moment. Overlapping the period of Chemical Evolution we have marked the period of Organic Evolution, up to the present day. This period of Organic Evolution is the one whose later part is recorded in the form of fossils. However, for its greatest part, beginning some time in the Archezoic period and extending through the Proterozoic period, there is no fossil record. This period of Organic Evolution of the soft-bodied living organisms, which left no fossils, constitutes by far the longer fraction of the period which we call Organic, or Biological, Evolution. Finally, at the very apex of the entire structure exists a point which we call the Evolution of Man. This consitutes only something like the last million years of geologic time, and it is clear that this represents a minute fraction of the time period with which we have to deal.

THE PRIMITIVE ATMOSPHERE

It should be noted that one of the prerequisites of all of the speculation about the origin of life is that there exists a means of gradually producing relatively complex organic substances by nonbiological processes. This question is susceptible to experimental investigation, and has been investigated by a variety of experimental scientists, with positive results. It is clear that in order to test any chemical process as a possible means of generating organic materials by nonbiological means, we must first know what the raw materials for these chemical processes must be. This, of course, entails some knowledge of the nature of the atmosphere of the primeval earth. This, in turn, requires a concept of the mode of formation of the earth and the solar system in which it exists. There have been a wide variety of hypotheses on this point. For example, Shapley in his recent discussion lists some fifteen different hypotheses with regard to the origin of the earth and the solar system (Shapley, 1958; Hoyle, 1955). In any case, one thing is common to all of the hypotheses, namely, that the earth did have a solid crust and some kind of a gaseous atmosphere. The question that is open to some discussion is whether that crust and atomosphere was primarily oxidizing or primarily reducing in character. In the former case, the dominant partners for all the atoms are oxygen atoms, while in the latter they are hydrogen atoms.

It is clear that no matter what concept one accepts for the origin of the earth, the atmosphere itself must have been made up of relatively simple molecules such as nitrogen, ammonia, possibly carbon dioxide, methane, hydrogen and the

like. A group of these molecules is shown in the first row of figure 2. There has been considerable discussion as to whether the oxidized molecules or the reduced molecules constituted the major portion of this atmosphere. It would appear that the present consensus favors the reduced group. In any case, experiments have been done with both types of atmosphere.

Random Organic Synthesis—the Beginning of Chemical Evolution

The agents which have been called upon to produce the initial transformations have nearly all been of the high-energy type—ultraviolet radiation, electrical discharge, radiation from radioactivity of earth-bound minerals, or radiation coming to us from outer space in the form of cosmic rays (Oparin, 1957). Since it appears that all of the concepts of the earth's formation involve the absence of molecular oxygen from its primeval atmosphere, the intensity of ultraviolet light which impinged on the surface of the earth in its early days was considerably greater than it is today. So, some of the earliest experiments designed to determine whether more complex organic molecules, containing carbon-carbon bonds, could be formed were done using the ultraviolet light as the source of the energy. They were described by Haldane as early as 1928 and they have been since checked in a variety of laboratories throughout the world. The primitive carbon compounds used in most of these experiments were already partly reduced, such as carbon monoxide, formic acid, or formaldehyde (Haldane, 1928, see 1954).

In 1951, in our laboratory, experiments were again instituted to determine the usefulness of high-energy radiations, such as those which might be derived from the natural radioactive materials present in relatively high concentrations in the primitive earth, or from cosmic radiations coming in from space (Garrison *et al.*, 1951). Here, the primitive carbon compounds were of the more oxidized type, strictly speaking, carbon dioxide. However, molecular hydrogen was also present in the experiments. The partial reduction of carbon from the completely oxidized form of carbon dioxide to a partly reduced form, such as formic acid or formaldehyde, was observed. In addition, new carbon-carbon bonds were apparently formed upon irradiation of aqueous solutions of such carbon compounds, leading to compounds such as oxalic acid and acetic acid, shown in the second row of figure 2.

Still more recently, beginning with the premise that the primeval atmosphere was of a reducing character, experiments were undertaken which were designed to test the effectiveness of electrical discharge in the upper atmosphere (a reduced atmosphere) to create materials more closely resembling those which are presently used in biological activities (Miller, 1955). Here the compounds used as starting materials were water vapor, methane, ammonia and hydrogen. These were primarily the reduced forms of each of the elements, that is, the elements of oxygen, nitrogen, carbon, attached only to hydrogen. When an electric discharge was passed through such a mixture, one indeed did get a large variety of more complex materials, particularly those known as amino acids, of which the simplest, glycine, is shown in the figure. It is perhaps worthwhile to point out that having reduced the one-carbon compound in the high-energy experiment and then formed a two-carbon compound by connecting two carbon atoms in the form of acetic acid, it was possible to demonstrate the generation of succinic acid, a four-carbon compound, resulting from the combination of two molecules of acetic acid, by irradiation with high-energy radiations such as those which one might obtain from radioactive materials. It is interesting to note that these latter experiments, as well as the first ones of 1951, were done using a cyclotron rather than natural sources of radioactivity because of the high intensities of ionizing radiation which could be obtained.

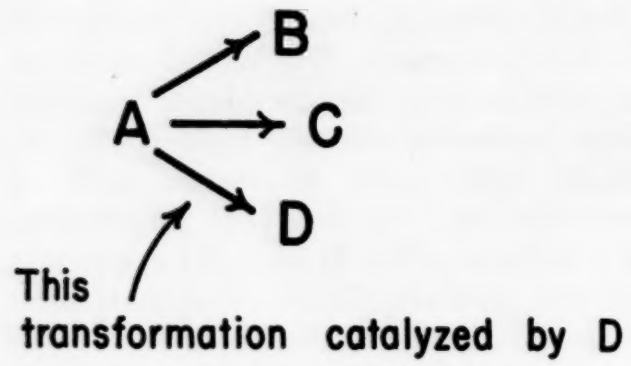

FIG. 3. Autocatalysis as a means of chemical selection.

One suggestion of a very different type was among the early ones used, since the elementary chemistry was already well known. This chemistry begins with metallic carbides (Oparin, 1957), which, on contact with water, will produce a variety of hydrocarbons such as methane and acetylene (the latter the familiar process used in the older miner's lamps). Some of these gases are of such a character that if they come into contact at high enough concentration with any of a variety of mineral surfaces, they will combine with each other to produce large, complex molecules, sometimes with very specific configurations (Natta, 1957). The metal carbides used to start such a process generally require high temperatures for their formation, and it will therefore be necessary to suppose either that the earth began very hot or that it has had at least some places in, or on, it that were hot enough to form such carbides and later deliver them to the cooler surfaces. There appears to be no geologic evidence for the primary existence of such carbides in the deep rocks.

Very nearly all of these processes which we have just described for the generation of new carbon-carbon and carbon-nitrogen bonds and the creation of more complex molecules from simpler ones are processes which depend upon the primary disruption of the simple molecule into an active fragment, followed by the random combination of those active fragments into something more complex. As the material present in the atmosphere and on the crust of the earth is gradually changed from the simple to the complex, these methods of transformation will not select between the simple and the complex and are just as prone to destroy by bond breakage the products of their initial activity as they are to create new ones. It is from here that we must now call upon some method of selective construction of molecules.

THE EVOLUTION OF CATALYSTS—ENZYMES

To do this we need only call upon the phenomenon of autocatalysis, well known to chemists (Calvin, 1956). This phenomenon occurs whenever the product of any chemical transformation has the property (catalytic) of influencing the rate of its own formation. It is somewhat surprising that this phenomenon was not long ago utilized in such discussions as these, since it is, in essence, the very fact and form of the mechanism of all selective evolutionary processes, namely, the selective superiority of any form to reproduce itself that will give rise to a transformation of material into that particular form.

Thus, it is easily apparent from figure 3 that of three possible transformations which A might undergo, namely, to B, to C or to D, it will undergo more frequently the transformation to D because D itself is a better catalyst for that transformation than it is for the others. Therefore, eventually the random processes which gave rise to A will not be quite so completely random in their further effectiveness. They will, in this case, tend to produce D rather than B or C from A. In general, the selective process of autocatalysis, and in higher evolution as well, is not an "all or none" process as we have just described it, but rather one of a matter of degree. Thus, the actual situation is more likely to be that all three substances, B, C and D, are all catalysts for their own forma-

tion, but the most efficient is the one which will eventually supersede the others.

It is of interest to examine how this sort of chemical selectivity might have functioned in the development of an extremely important biological material which is widely distributed today. This class of material is represented by that which gives the red color to blood cells and the green color to leaves. It constitutes a very important class of organic substances known by the name of porphyrins. We have already seen how the simple precursors to porphyrins, succinic acid and glycine, might be formed (and, indeed, were formed) by a random process from the primeval carbon-containing molecules. Now we have available to us a method of tracing the route by which these two precursors eventually become porphyrins in present-day living material, and some of the essential steps in that series are shown in figure 4 (Shemin, 1954).

Underneath the center sequence are indicated, by arrows, a number of possible points where the catalytic function of iron might play a role. For example, iron may play a part in the combination of succinic acid and glycine to start with, or in the decarboxylation of the primarily-formed keto adipic acid which is the second compound in the center row, or, finally, in one of the several steps which are necessary to convert the delta-amino-levulinic acid into the macrocyclic, or large ring, compound known as protoporphyrin No. 9 at the right-hand end of the figure. We already know that a number of these steps are definitely catalyzed by iron, but what is more important is that some of them are much more readily induced to go by the iron ion after it has been encased in the porphyrin No. 9, as shown by the dotted circle in the porphyrin, than they are by the bare iron ion. Such an iron porphyrin is a much better catalyst for several of the steps leading to its own formation than is the bare iron ion itself. Furthermore, it is almost certainly a better catalyst for the conversion of the levulinic acid toward the porphyrin than for the competitive conversion of the glycine back toward the carbon dioxide whence it came. Thus, it is clear that the route from succinic acid and glycine to the porphyrin, once the porphyrin has been formed, will be greatly facilitated by the incorporation of iron into it, thus bringing a good deal more of the succinic acid and

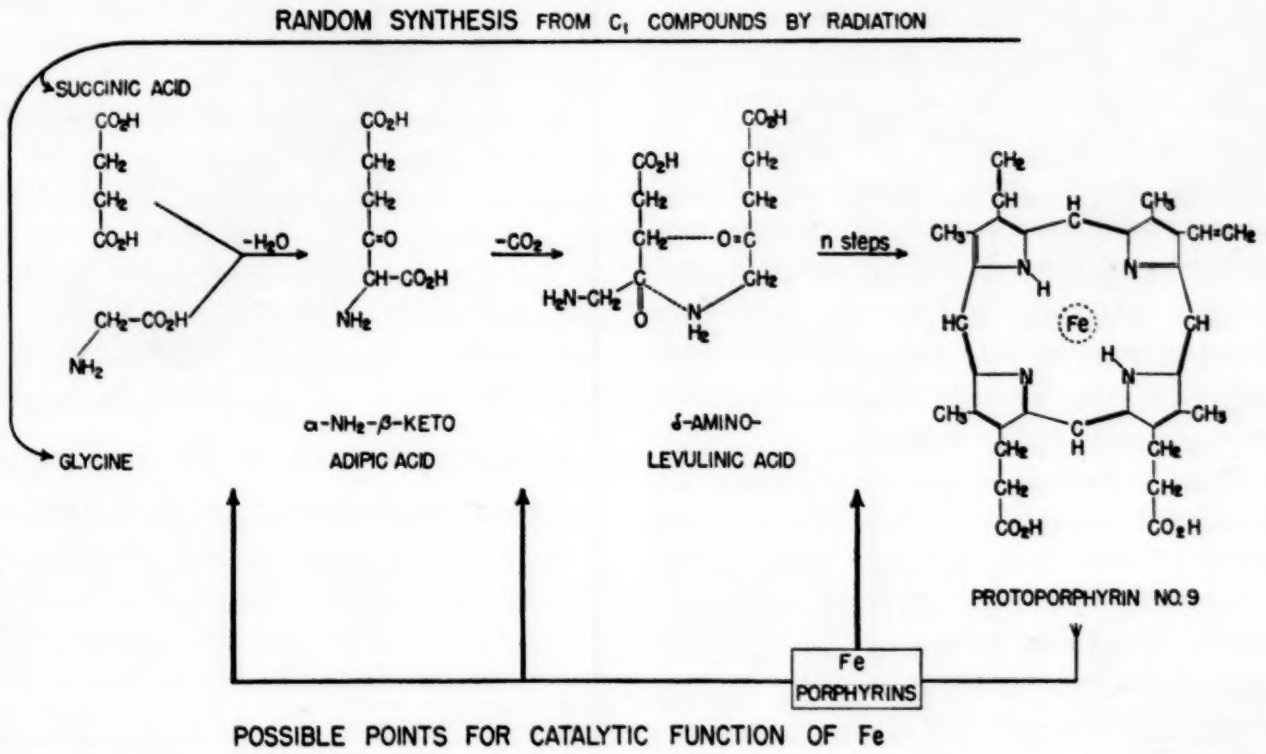

FIG. 4. Biosynthesis of porphyrin and the evolution of the catalytic functions of iron.

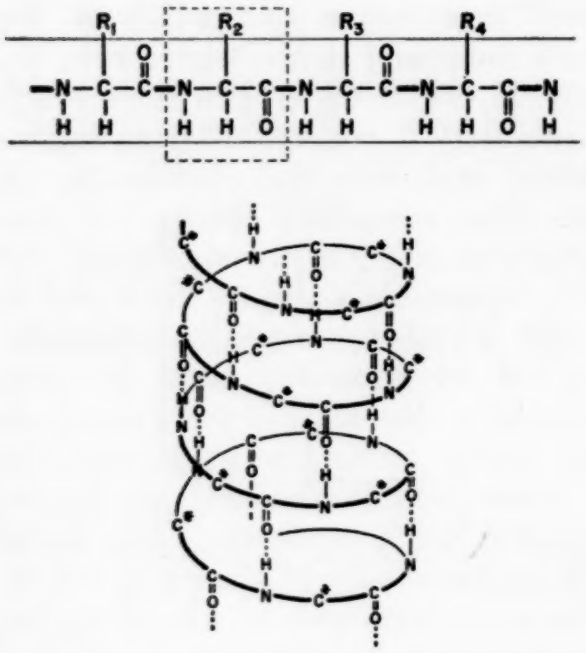

FIG. 5. Protein structure: simple structural principles and variety of chemical reactivity.

glycine into this particular, and important, form.

The process of the development of the catalytic function of iron does not and has not stopped at this point. When the iron compound is built into a protein (a macromolecule made by combination of many amino acids), its catalytic efficiency may be increased still more. Beyond this, the variety of chemical changes in which it may assist can be increased and the efficiency of its function diversified.

Other atoms and groups of atoms having rudimentary catalytic powers can be developed into the highly efficient and specific catalysts which we know as enzymes in even the simplest of modern organisms, by exactly analogous processes of chemical selection by autocatalysis. And it is almost certainly no "accident" that these enzymes are all proteins. For it is only the proteins as a class (fig. 5) that offer the combination of simplicity of formation (peptide bond) with practically an infinite variety of chemical function (R Groups) (Crick, 1958).

FROM CHAOS TO ORDER—MOLECULAR CRYSTALLIZATION

So far, all of the processes of which we have spoken have been described as taking place in a rather dilute solution, with the molecules randomly arranged. The development of a complex material under such circumstances could not go very far. Two further stages of change seem to be required: (1) the ordering of the molecules in some rather specific array and (2) the concentration of the formed substances into relatively small packages. Which of these two processes took place first, or whether they were indeed successive or simultaneous developments, is hard to say. However, that both of them took place we do know, and that there exist mechanisms for each of these processes to take place we can also demonstrate in the laboratory.

One type of molecule, for example, with which we are constantly dealing, both in the laboratory and as a result of present-day biological action, is the large,

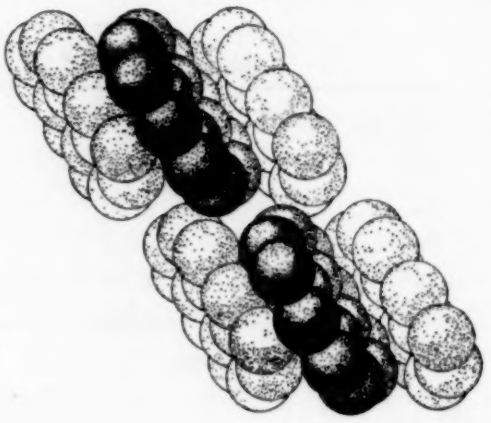

FIG. 6. Packing of flat molecules in a crystal.

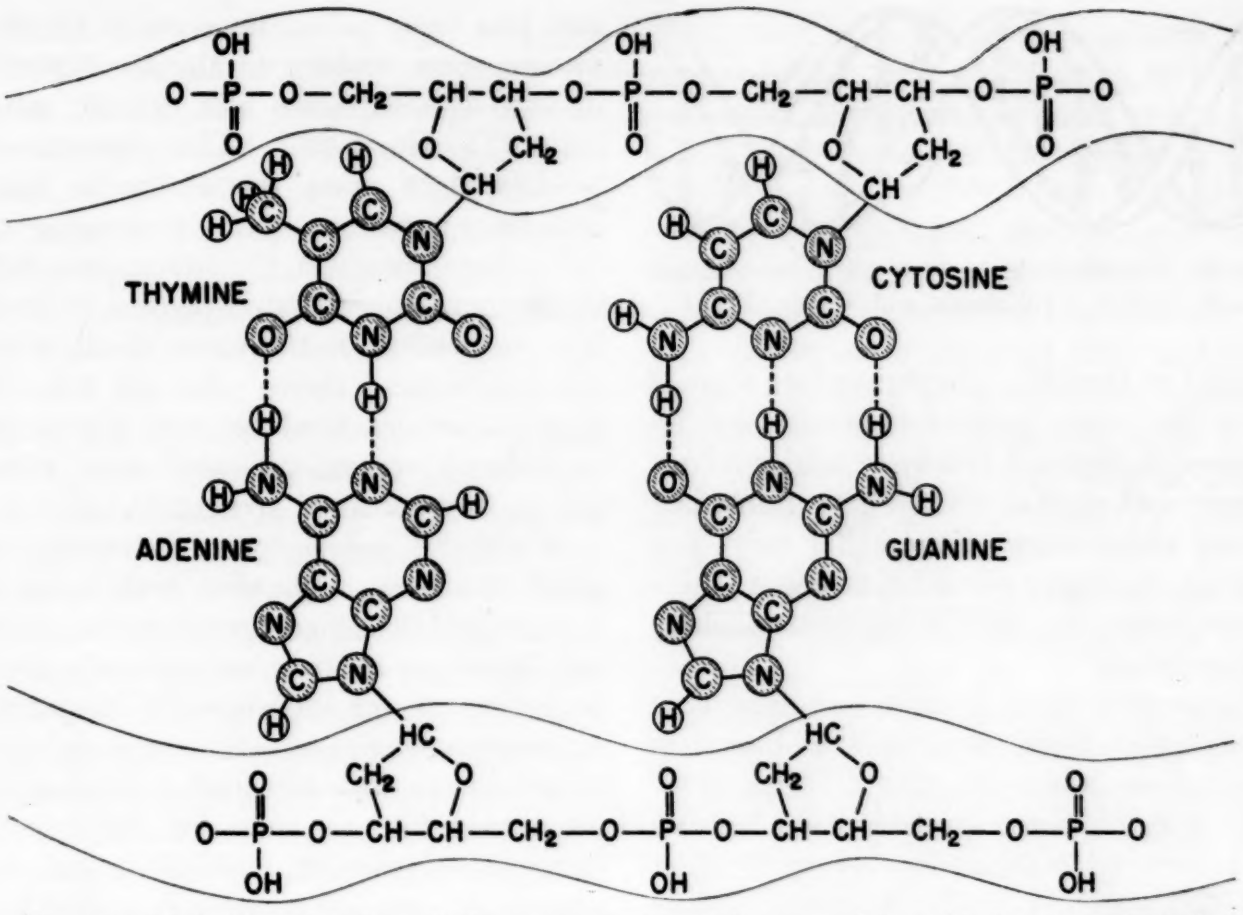

FIG. 7. Molecular drawing of the components of desoxynucleic acid (genic material).

flat so-called aromatic molecule. The porphyrin that we have just mentioned constitutes one such, and there are many others. Such molecules, when they reach a certain minimal level of concentration in aqueous solution, will tend to drop out of that aqueous solution. However, they will not drop out in a random way. Because of their peculiar flat shape, they will tend to drop out in an arrangement in which the molecules are piled one on top of the other, much like a pack of cards. If one throws a pack of cards in the air and allows them to fall out of the air, onto the floor, it is unlikely that they will fall standing on edge. They will, of course, practically all (if not all) land flat, one on top of the other, because of their peculiar shape. This kind of phenomenon illustrates the way in which large, flat molecules tend to come out of solution in crystals. Figure 6 shows a diagrammatic drawing of a simple crystal of a rather simple flat molecule, and here you can see the result of the tendency of such molecules to pile up, one on top of the other (Calvin, 1958).

I use this particular type of example with a purpose in mind. Much of the information that present-day living organisms carry with them and can transmit to their offspring in the form of genetic material is made up, or seems to be contained, in an ordered array of such flat molecules as this. The additional feature that one must add to this piling-up of flat molecules is that they are not independent, as the group of cards was, but that they are all tied together along an edge, as though the group of cards were tied together by a double string. The example of the flat units that we will use here will be the pairs of complementary bases as they are found in desoxynucleic acid (DNA), the material of which the chromosomes are constructed. Figure 7 shows these pairs as they occur: thymine paired with adenine cytosine paired with guanine by virtue of their peculiar structure which places certain of their hydrogen atoms between oxygen and/or nitrogen atoms on the complementary molecule. These four molecules, and one other very closely

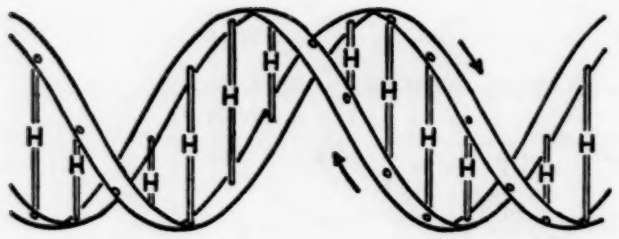

FIG. 8. Double helix model for desoxynucleic acid (DNA) (Watson and Crick, 1953).

related to thymine, are themselves formed from the same primitive precursors described in figure 2 (succinic acid, glycine, formic acid, carbon dioxide and ammonia). Along either edge of these flat units you can see the sugar-phosphorus chain which corresponds to the string-card analogy above noted.

Because of their peculiar aromatic type of structure these base pairs will tend to line up one above the other. This tendency will, of course, be increased because they are not independent of each other but tied together by the sugar-phosphate ribbons. In actual fact, the structure of desoxynucleic acid (DNA) seems to be such a flat piling up of the nucleoside bases with the sugar-phosphate ribbons twisted in a spiral around the outside as shown in figure 8.

CONCENTRATION AND LOCALIZATION—THE FORMATION OF A CELL

The other aspect noted, which must obtain, is the concentration into relatively small volumes in space of these organic materials, leading ultimately to the formation of cells as we now know them as the units of living matter. It is at this point that our information and our analogies are most diffuse. However, a number of physical-chemical processes have been called on to participate in the development of the living cell. First among these is the appearance of surface layers, or boundary layers, such as one sees in a soap bubble. Here we are quite familiar with mechanisms for producing a relatively stable and well-defined boundary layer between two phases. Another phenomenon which is much less familiar to us, is known by the name of coacervation and has been called upon as a primary phenomenon leading to the development of local concentration and cellular structures (Oparin, 1957). This phenomenon is dependent upon the ability of giant molecules in water solution to separate out from the dilute water solution into relatively more concentrated phases, or droplets, suspended in the more dilute water solution around them. Beyond this, the giant molecules tend to pack themselves in ordered arrays, provided they, themselves, have ordered structures.

While the knowledge of interaction of giant, or macro, molecules, both synthetic and natural, has made great strides in the last decade or two, we are still only at the beginning of our investigation of systems of macromolecules of sufficient complexity to provide us with the kind of information we would like to have for the present purposes. However, it seems to me, that already enough information is available to us to be able to say with confidence that the basic kinds of physical-chemical processes upon which we will have to draw in order to describe the evolution of the cellular structure are already at hand.

THE SELECTION OF THE PILOT—DNA

That characteristic which is most frequently invoked as the prime attribute of living material is the ability to reproduce and mutate. Very frequently in discussions of the origin of life, attempts are made to define that certain point in time before which no life existed and after which we may speak of "living" things as that point at which an organic unit, be it a molecule or something bigger, came into existence which could generate itself from existing precursors and which could sustain and propagate a structural change. Since we can be confident that genetic information is transmitted today in the environment of a cell by the chemical substance known as desoxynucleic acid (DNA), it seems reasonable to seek in this structure the clues to the mutable self-reproducing molecule, or unit (Watson and Crick, 1953).

It should be pointed out that while we are in the habit of thinking of the nucleoprotein molecules which constitute the chromosomes and viruses (DNA and RNA, ribonucleic acid) as containing all the information required to produce its entire organism, this is not strictly true. These structures may be said to contain information only in relation to, and exhibitable through, a proper environment. Thus, it is possible to keep a bottle containing only virus particles (say DNA) indefinitely, just as any other organic chemical, and the question of whether it is alive or not would not arise. The moment these particles find themselves in a suitable organic medium (such as may be found in any of a variety of cell cytoplasms), this information makes itself apparent and the virus multiplies. There are thus other constituents in the modern cell which contain indispensable information but under direction of the nucleoprotein of the chromosomes.

While a mechanical model (Penrose and Penrose, 1957) operating in one dimension has been described which is able to control the behavior of a mixture of precursor units as it goes on to form a larger material, it is only in recent months that the ability to separate the controlling information-carrying units from the energy-transforming units and to demonstrate that information can indeed be carried by the desoxynucleic acid particles has been achieved (Kornberg *et al.*, 1958a, b, c; Grunberg-Manago *et al.*, 1955).

The DNA, or chromosomal material, is made up of a linear array of only four units, represented in figure 7 by the four bases (adenine, thymine, cytosine and guanine) and the controlling information about the organism (at least a modern post-Cambrian cell) is contained in some kind of linear array of these units. In the past few years it has been possible to isolate from various living organisms, particularly bacteria, a catalyst (enzyme), which, when placed into a solution containing all four of these units in an active form, that is, as their triphosphates, was able to induce their combination into some particular linear array of bases to produce a particular variety of desoxynucleic acid. Which particular DNA was formed depended entirely upon the presence of a very small amount of so-called "starter" which had to be added to this mixture. If this starter were obtained from one type of cell, that particular type of desoxynucleic acid would be formed; from some other type of cell, another type of DNA would appear (Kornberg *et al.*, 1958d, e, f).

Still more recently, it has been possible to make a synthetic desoxynucleic acid, consisting of only two of these bases, in particular the thymine and adenine (Schachman, 1958). This synthetic DNA presumably has no counterpart in nature today, and yet when this synthetic material is given as a "starter" to the reaction mixture, as previously described, that particular two-base desoxynucleic acid, made up of only thymine and adenine, is produced. The catalyst which is able to do this seems to be the protein-like material constructed of amino acids in the way proteins usually are. Although little is yet known about the nature of this catalyst (enzyme) it will almost certainly be related to the simpler compounds and elements whose more primitive catalytic abilities constitute the basis for its action. This is exactly analogous to the relationship between the catalytic ability of simple iron ions and that of the highly effective iron proteins, as described in figure 4.

We have thus traced a path from the primitive molecules of the primeval earth's atmosphere through the random formation of more or less complex organic molecules, using the available energy sources of ultraviolet light, ionizing radiation or atmospheric electrical discharge, through the selective formation of complex organic molecules via autocatalysis, finally, to the information-transmitting molecule which is capable of self-reproduction and variation. During the course of this process we have, naturally, made use of the or-

ganic materials which have been produced in high-energy form via these various energy-yielding routes. In addition, somewhere, either during the course of this Chemical Evolution, or perhaps succeeding it, a system was evolved in which the concentration of the reaction materials was retained in a relatively small volume of space, leading to the formation of cellular structures.

Plotting the Course—Biosynthetic Pathways

During this entire course we have made use of the randomly-formed molecules, followed by a chemical kind of selection. The ultimate production of the information-carrying molecules depended upon the preformed presence of their constituent units, for example, nucleoside triphosphates or "active" amino acids. It is clear that as the efficiency of transformation is increased by chemical or early biotic evolution, all of these precursors will have been used up, and a mechanism will have to be devised for the regeneration of those precursors by more specific chemical routes than those originally used. We can see these very specific biosynthetic routes in the living organisms of today.

The last ten to twenty years, since the application of tracer techniques particularly, has exposed to us the wide variety of relatively complex biosynthetic sequences, an illustration of which we had in figure 4, leading to the porphyrin, and which today appear to be a sequence of reactions didected toward a particular end. The usefulness of any intermediate step does not become apparent until the final product is formed. Such sequences, on an organismic level, have led to a variety of teleological theories about the nature of evolution. However, on the molecular level it is possible to see the way in which complex, apparently directed, biosynthetic sequences arose by the operation of the ordinary laws of physics and chemistry, including the idea of autocatalysis as the basis of selection.

This was pointed out by Horowitz some years ago when he recognized that once having formed a useful material into an "organism," which could transmit its information to its offspring, this process would continue so long as precursors were available for this "organism" to use for its reproduction (Horowitz, 1945). However, eventually it was clear that one or another of these precursors would become exhausted. That particular organism which could adapt itself by a random variation to make the missing precursor from molecules which still remained available to it would, of course, survive, provided the knowledge of how it was done could be transmitted; all the others would die out. Thus, we would now have lengthened the chain of synthesis by one step, but in a backward direction toward the simpler precursors. By extrapolating this back, eventually to carbon dioxide, one can get the very complex and what appear to be totally directed syntheses from the very simplest of all carbon compounds.

The Ultimate Fuel—Photosynthesis

We have remaining one additional attribute which is always associated with living material and which is very frequently called upon as a prerequisite to life, namely, the ability to use energy-yielding chemical reactions to create order out of disorder. Ultimately, of course, the large-scale evolution of living organisms to the extent that we are now familiar with it could not take place until the invention of photosynthesis, that is, the coupling of the ability of certain molecules to absorb solar energy, to the ability of certain other molecules to use this energy for the synthesis of the necessary structures. Here it is almost certain that the ability to synthesize had long been evolved before the ability to couple the absorbed solar energy to those synthetic reactions was discovered. The use of porphyrins by nonphotosynthetic organisms is widespread, and almost certainly random

variation in structure led to the discovery that small changes in the porphyrins, leading to the construction of chlorophyll and its use in transmitting energy for biosynthesis, led to the invention of photosynthesis (Calvin, 1957).

Strictly speaking, the primitive synthesis, making use of ultraviolet light or ionizing radiation, is a form of photosynthesis and in all probability there existed a parallel evolutionary development of this kind of energy-conversion process. In fact, modern work by physical chemists on the effect of the far ultraviolet light on some of the simple molecules we spoke of earlier as constituting the primitive atmosphere of the earth, has demonstrated experimentally the feasibility of the idea of the conversion of water (H_2O) into hydrogen (H_2) and oxygen (O_2) (Norrish, 1958). It has even been possible to demonstrate the conversion of carbon dioxide (CO_2) into carbon monoxide (CO) and oxygen (O_2) using sunlight of such a high energy that very little of it penetrates down very far into the present earth's atmosphere (Mahan, 1958).

Whether the conjunction of the use of visible light-absorbing substances, such as the porphyrins, with the biosynthetic demands of the more highly evolved chemical systems, took place before or after the appearance of what we would today call living organisms matters little for the purposes of our present discussion, important though the question may be. It seems quite clear, however, that these two parallel lines of development did meet, as mentioned earlier, giving rise to the very efficient energy conversion processes resembling those which we know today. It is not unlikely that the final step in the development of modern photosynthesis, namely, the evolution of oxygen, did not take place until relatively late in the sequence of events (Calvin, 1957). For example, we do have organisms today which are capable of using solar energy, via the agency of porphyrin-type molecules, but using other methods of taking care of the oxygen by combination with suitable reducing agents such as hydrogen. These appear in the form of photoreducing organisms such as the photosynthetic bacteria. They dispose of the oxidizing fragment of the water molecule, made by the absorption of light, by combining it with whatever reducing materials may have been present in the primitive atmosphere. It is not until the higher green plants appear that we find the ability of disposing of this oxygen back into the atmosphere as molecular oxygen.

The Fossil Record—Man's Place

With this biological discovery the stage was set for the enormous development of living organisms on the surface of the earth as we know them today. From here on, the fossil record is quite complete and there is little point for us to pursue in detail the ascent and divergence of life, leading ultimately to mankind in the last million years (Simpson, 1950; Dobzhansky, 1955).

It is perhaps worthwhile at this point to try and assess the amount of time which may be allotted to each of the major kinds of operations, or sequences of events, which we have outlined as leading ultimately to cellular life as we know it on the earth today, and man. From the very beginning the fossil record contains evidence of very nearly all of the major subkingdoms, or phyla, of life today. This fossil record, which is some 500 to 1,000 million years old, thus indicates that by the time life was sufficiently well developed to leave a fossil record it had already manifested itself in very nearly all of the major types of forms which we now recognize. This time period constitutes something less than one-quarter of the entire habitable life span of the earth. Thus we have some two or three billions of years during which we can pursue the process of Chemical Evolution, overlapping with that part of the evolution of cellular life (biotic evolution) which was unable to leave a record of itself in the

rocks. This is an extremely long period of time which gives us ample opportunity for the enormous numbers of trials and errors which would be required to develop all the possible molecular processes and combinations which must have been tried.

Undoubtedly many different information-molecular species had a birth, a life span and a death, much as we now see in the fossil record for the higher forms. One (DNA), or only a few, of these closely related information-carrying molecules, or molecular species, eventually superseded all the rest because of the particular structural, chemical and dynamic properties of this arrangement of atoms, i.e., stability, template or complementarity quality, mutability and others as yet not defined. It was from this (these) that the present-day organisms have developed, thus providing a basic similarity in all living processes as we know them today.

The determinism in the arrangement of a system increases with the number of trials (events) that can occur in reaching it. On the molecular level, where the number of changes occurring per second is high, predictability with regard to what will happen in a given situation to a group of molecules amounts to certainty. For example, pressure maintained by bombardment of molecules on the walls of a vessel containing a certain number of gas molecules at a specified temperature is quite predictable. While we cannot specify the particular molecules which will strike the wall at any one time, we can be quite sure that in a given period of time a certain number will do so.

At the other extreme, the segregation and recombination of genes which may take place in the formation of a new individual by (geological) modern genetic mechanisms appears as a completely undetermined, or random, choice, since only a single event is involved. However, among a group of organisms of a given species there will be a predictable distribution of properties at a given time under a specified set of conditions.

On the other hand, coming up from molecules and molecular aggregates, we will reach a stage, probably after the invention of cellular heredity based on nucleic acid, when the number of events (rate multiplied by time available) will those that have appeared will ensue.

Therefore, while an indeterminism exists with respect to the character of any individual living thing and a limited indeterminism exists with respect to species, the time element is so great and the amount of genetic material which has been cycled through the sequence of birth, growth, development and death is so enormous (Swanson, 1957) that the certainty of the occurrence of cellular life as we know it on the earth today seems assured, given the initial starting conditions.

A most convincing demonstration that such a sequence of events, leading from nonliving matter to life, could, and probably did, take place, would be an experiment in which a system of organic material, called alive by most biologists, is produced through the agency of no other life save the hand of man (Reichenbach, 1951). And already today there is serious discussion as to whether some of the experiments performed in the last year might not fulfill these conditions.

It thus appears that man is a rather late and highly developed (perhaps the most highly developed) form of that organization of matter which we call living on the surface of the earth, and which is the result of the peculiar and special environmental situation provided on the surface of the earth since its formation some five billion years ago. We have known for some time now that the earth is the number three planet in orbit around a rather ordinary star on the edge of one of the minor galaxies of the universe. Thus, presuming life to be a unique occurrence limited to the surface of this one rather trivial (in terms of mass, energy, position, etc.) planet in the universe, man, though an impressive representative of the state of matter called living, is not viewed as a major cosmic force.

Life on Other Planets?—A Cosmic Influence

Now man is about to send back into space some bits of the dust from whence it (the dust) originally came. This return will be in the form of machines, and eventually of man himself. It is thus not only timely but more significant than ever before to ask again the question: What are the probabilities that cellular life as we know it may exist at other sites in the universe than the surface of the earth? In view of the chemistry of carbon (and a few of its near neighbors) and the consequences this has given rise to in the environment to be found on the surface of the earth, all that is required to answer, or to provide some kind of answer, to this question would be an estimate of the number of other sites, or planets, in the universe which might have the environmental conditions within the range to support cellular life as we know it on the surface of the earth.

Here we follow Shapley (1958; see also Hoyle, 1955) who begins his calculation with an estimate of the minimum number of stars which might be in the universe. His minimum calculation, based upon the best telescopes we have today, is of the order of 10^{20}, that is, one with twenty zeros after it. The next step is to determine what fraction of these stars will have a planetary system. To make this estimate one must have some concept of how our planetary system was generated. Here there are many theories and Shapley lists fourteen or fifteen of them. However, there are a number of conditions which must be fulfilled and these, taken together with the fact that the universe has been expanding, and some five billion years ago when the planetary systems were formed the universe must have been much more crowded than it is today, give the figure one in a thousand as a very conservative estimate of the number of stars which have a planetary system.

The next question which Shapley attempts to answer is what fraction of these planetary systems will contain a planet at approximately the correct distance from its star such that it will have a temperature variation compatible with the cellular life that we are seeking to determine. Here, again, we arrive at the very small, and what we consider conservative, figure of only one of these planetary systems in a thousand as containing a planet at approximately the right distance from its sun. The next circumstance which must be fulfilled is that of size. Again, the planet must be of the correct size to retain an atmosphere and not be too large. Here Shapley gives the figure of one in a thousand as a conservatively small fraction.

Finally, of all of these planets which are the correct size, which of them will have the proper atmosphere containing carbon, hydrogen, nitrogen and oxygen, which will be required to give rise to cellular life as we know it on earth? The answer is, again, the very conservative number of only one in a thousand.

Thus, we have four factors of one-thousandth by which we must multiply our figure of 10^{20} (the minimum number of stars which might be in the universe). This leads to the number 10^{8}, that is, one hundred million, as the number of habitable planets to be found in the universe. Remember that this calculation is limited to those planets which will have conditions within the range compatible with cellular life, based on carbon, as we know it on the earth. This does not include such systems, which conceivably we can imagine, based on other elements, such as silicon, or nitrogen, or perhaps even on anti-matter. Such worlds and such systems may very well exist. However, these are not included as possibilities in this calculation. We have already cut down the limits by our four factors of one-thousandth, leading to something of the order of 100,000,000 planets which can support cellular life as we know it on the surface of the earth.

Since the time element seems to be about the same for all parts of the universe, namely, something greater than

five billion years, it would appear that we can be reasonably certain that these hundred million other planets will indeed have cellular life on them. Since in the course of the chemical and biotic evolution the appearance of man on the surface of the earth has occupied only a very small fragment of time, namely, only one million years of the five billion, it is clear that we may expect to find cellular life, and perhaps precellular life and posthuman life, in many of these other planets. The one million year period, given to the evolution of man on earth, constitutes an extremely small element of this long period of time and an uncertainty of this order could, and almost certainly does, exist in the status of the evolutionary sequence elsewhere in the universe.

We can thus assert with some degree of scientific confidence that cellular life as we know it on the surface of the earth does exist in some millions of other sites in the universe. This does not deny the possibility of the existence of still other forms of matter which might be called living which are foreign to our present experience.

By answering our second question in this way, we have now removed life from the limited place it occupied a moment ago, as a rather special and unique event on one of the minor planets around an ordinary sun at the edge of one of the minor galaxies in the universe, to a state of matter widely distributed throughout the universe. This change induces us to re-examine the status of life on the surface of the earth. In doing so we find that life on the surface of the earth is not a passive, existing thing, but actually changes and forms the environment in which it grows. The surface of the earth has indeed been completely transformed in its character by the development of the state of organization of matter which we call life. Furthermore, it is undergoing another, and perhaps a more profound transformation, by one representative, or manifestation of that organization of matter, mankind.

Man in Space—the Next Step

Now that man has the capability of taking his machines and himself off of the surface of the earth and of beginning to explore outer space, there is no reason to suppose that life, and man as its representative, will not transform any planet, or any other astral body upon which he lands, in the same way, and perhaps even in a more profound way, than he has transformed the surface of the earth. For example, it might suit him in the future to change the course of the orbit of the moon and it seems within the realm of possibility that he should be able to do so. When we realize that other organisms may be doing similar things at some millions of other regions in the universe, we see that life itself becomes a cosmic influence of significant proportions, and man, as one representative of that state of organization of matter, becomes a specific cosmic influence himself. Thus have we come to a complete inversion of our view of the place of life and of man in the universe from a trivial to a significant cosmic influence. And we have come to this view entirely upon the basis of experimental and observational science and scientific probability.

Man's adventure into space, which is about to begin, is not merely a flexing of muscles—a demonstration of strength. It is a necessary aspect of evolution and of human evolution in particular. It is an activity within the capability of this complex organism, man, and it must be explored as every other potentially-useful evolutionary possibility has been. The whole evolutionary process depends upon each organism developing to the greatest extent every potential.

Acknowledgment

I would like to take this opportunity to express my thanks to my colleagues on the Berkeley Campus for the many varied and stimulating discussions we have had on this subject. I would particularly like to mention several with whom I had the opportunity to discuss many of the

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Literature Cited

Calvin, M. 1956. Chemical evolution and the origin of life. American Scientist, **44**: 248.

——. 1957. Evolution of enzymes and the photosynthetic apparatus. University of California Radiation Laboratory Report UCRL 3915.

——. 1958. From microstructure to macrostructure and function in the photochemical apparatus. Brookhaven National Laboratory Biology Conference 11 (in press).

Crick, F. H. C. 1958. On protein synthesis. Soc. Exptl. Biol. (G. B.). Symp. "Biological Replication of Macromolecules," **12**: 138. Cambridge University Press.

Darwin, C., and A. R. Wallace. 1858. On the tendency of the species to form varieties and on the perpetuation of the species by natural means of selection. Journ. Proc. Linnean Soc. London (Zoology), **3**: 35–62.

Dobzhansky, T. 1955. Evolution, genetics and man. John Wiley & Sons, Inc., New York, New York.

Garrison, W. M., D. C. Morrison, J. G. Hamilton, A. A. Benson and M. Calvin. 1951. The reduction of carbon dioxide in aqueous solutions by ionizing radiation. Science, **114**: 416.

Grunberg-Manago, M., P. J. Ortiz, and S. Ochoa. 1955. Enzymic synthesis of nucleic acid-like polynucleotides. Science, **122**: 907.

Haldane, J. B. S. 1954. Collection of essays on The origin of life. New Biology, No. 16. Penguin Books Ltd., London, England.

Horowitz, N. 1945. On the evolution of biochemical synthesis. Proc. Nat. Acad. Sci., **31**: 153.

Hoyle, F. 1955. The nature of the universe. Mentor Books, New York, New York.

Kornberg, A. (*et al.:* M. J. Bessman, I. R. Lehman, J. Adler, S. B. Zimmerman, and E. S. Simms). 1958a. Enzymatic synthesis of deoxyribonucleic acid. III. The incorporation of pyrimidine and purine analogues into deoxyribonucleic acid. Proc. Nat. Acad. Sci., **44**: 633.

—— (*et al.*). 1958b. Enzymatic synthesis of deoxyribonucleic acid. IV. Linkage of single deoxynucleotides to deoxynucleoside ends of DNA. Proc. Nat. Acad. Sci., **44**: 641.

——. 1958c. Enzymatic synthesis of deoxyribonucleic acid. Abstracts of 134th National Meeting of the American Chemical Society, Chicago, Illinois, September 1958. Abstract 21C.

—— (*et al.*). 1958d. Enzymatic cleavage of deoxyguanosine triphosphate to deoxyguanosine tripolyphosphate. J. Biol. Chem., **233**: 159.

—— (*et al.*). 1958e. Enzymatic synthesis of deoxyribonucleic acid. I. Preparation of substrates and partial purification of an enzyme from *E. coli.* J. Biol. Chem., **233**: 163.

—— (*et al.*). 1958e. Enzymatic synthesis of deoxyribonucleic acid. II. General properties of the reaction. J. Biol. Chem., **233**: 171.

Mahan, B. 1958. Private communication.

Miller, S. L. 1955. Production of some organic compounds under possible primitive earth conditions. J. Am. Chem. Soc., **77**: 2351.

Natta, G. 1957. Stereospecific catalysis and stereoisomeric polymers. Speech at opening conference, XVI Congress of Pure and Applied Chemistry, Paris, France.

Norrish, R. G. W. 1958. Some isothermal reactions of free radicals studied by kinetic spectroscopy (Liversidge Lecture). Proc. Chem. Soc. London, Sept. 1958, 247.

Oparin, A. I. 1957. The origin of life. 3rd Eng. Ed. Oliver and Boyd, London, England.

Pauling, L., and H. Itano. Molecular structure and biological specificity. Amer. Inst. Biol. Sciences, Washington, D. C., Publication No. 2.

Penrose, L. S., and R. Penrose. 1957. A self-reproducing analogue. Nature, **179**: 1183.

Reichenbach, H. 1951. The rise of scientific philosophy. University of California Press, Berkeley, California, 202.

Sears, P. 1950. Charles Darwin. Charles Scribner's Sons, New York, New York.

Schachman, H. 1958. Private communication.

Shapley, H. 1958. Of stars and men. Beacon Press, Boston, Massachusetts.

Shemin, D. 1954. Biosynthesis of porphyrins. Harvey Lectures **50**: 258.

Simpson, G. G. 1950. Meaning of evolution. Yale University Press, New Haven, Connecticut.

Swanson, C. P. 1957. Cytology and genetics. Prentice-Hall, Inc., Englewood Cliffs, New Jersey. Chapter 12.

Watson, J. D., and F. H. C. Crick. 1953. General implications of the structure of DNA. Nature, **171**: 964.

Winchell, A. 1870. Sketches of creation. Harper & Brothers, New York, New York.

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EVOLUTION IN THE GENUS *GEUM*

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INTRODUCTION

The genus *Geum* of the family Rosaceae consists of about 56 species widely spread over the world except in the tropics. All species are perennial herbs growing in more or less mesic conditions. More than fifteen years' study of this genus by the present author have shown that this genus is a polyploid complex of which the various species represent different stages of evolution, and show interesting relationships.

In spite of great morphological differentiation on different polyploid levels the crossability between different species is surprisingly high, permitting cytogenetic analysis of numerous interspecific hybrids. *Geum* presents many interesting general problems concerning the role of geographical and ecological isolation as well as hybridization and polyploidy in the process of speciation. Some of the more interesting results of published (Gajewski, 1948, 1949, 1950, 1952, 1953, 1955, 1957) as well as unpublished results will be presented here.

THE POSITION OF *GEUM* IN THE FAMILY ROSACEAE

The species of *Geum* are rather well characterized and are easy to separate except for a few recent groups that show intensive hybridization or introgression, chiefly in areas where the original vegetation has been disturbed by man. On the other hand, the separation of *Geum* from related genera has been much discussed among taxonomists without any general agreement. The results of cytogenetic analysis have clarified to some extent this homologous, it seems more reasonable to species is an old polyploid complex in which different genome combinations represent distinct groups of more or less related species. The separate groups often differ very conspicuously in morphology and geographical distribution. They have probably arisen in different times. This was the cause of the separation of several groups of species in as many as six or even eight different genera (Rydberg, 1898, 1908–1916; Bolle, 1933; Jezupchuk, 1941). As the hybrids between some of these groups are still partially fertile and some of their genomes are homologous, it seems more reasonable to keep them together in one genus subdivided in many subgenera. This classification, I think, gives a better insight into the genetical relationships of the taxa studied, whereas splitting them into numerous genera obscures phylogenetic relationships.

The genus *Geum* (sensu lato), with the closely related but clearly separate genera *Waldsteinia* and *Coluria,* belongs to the tribe Geeae. This tribe, with the tribes Cercocarpeae (genus *Cercocarpus*) and Dryadeae (genera *Purshia, Cowania, Fallugia* and *Dryas*), forms a separate subfamily, Dryadoideae of the rose family. This subfamily is clearly separated from the related subfamily Rosoideae by having orthotropous ovules, and represents a separate evolutionary line.

The origin of the subfamily Dryadoideae is probably so old that the first steps of its evolutionary history can only be guessed. It seems very probable that the woody genera of the tribes Cercocarpeae and Dryadeae (except perhaps *Dryas*) confined to Western North America are the present day survivors of an old primitive group of Dryadoideae. They show now, of course, many derived characters ac-

FIG. 1. Diagram showing the relationships of the genera and subgenera in the subfamily Dryadoideae. The dark patches represent approximately the relative size of the different taxa. The outer circles show basic chromosome numbers and the type of the structure of the styles.

quired during their long evolution, as for instance the reduction of the number of pistils to one per flower in *Cercocarpus*. However, their restricted distribution, woody habit, long persistent and pennate styles adapted to wind dispersal seem to be more primitive than the herbaceous habit and styles specialized for transport by animals which are characteristic in most Geeae. All species of these two tribes, including *Dryas*, have the basic chromosome number x = 9 and only diploid species are known, whereas in the widespread tribe of Geeae with only herbaceous plants the basic chromosome number is 7 and polyploids are very numerous. A case of very curious parallel evolution seems to have occurred in the related subfamily Rosoideae with anatropous ovules. The tribe Kerriaeae with few, mostly monotypic, genera geographically restricted to the East and West shores of the Pacific like *Lutkea, Gillenia, Neillia, Pentactina* or *Stephanandra*, has also the basic chromosome number 9 with only diploid species known. The other tribes of this subfamily like Roseae, Potentilleae or Sanguisorbeae are mostly herbaceous with the basic chromosome number x = 7, and most genera are widespread in the Northern Hemisphere with numerous polyploid species. It seems thus that the tribes in both subfamilies with the basic chromosome number 7 are the youngest. Why the evolution from the shrubby to the herbaceous habit was

often connected with the change of the basic number from 9 to 7, and why the 7-chromosome genera have wider distribution, have formed numerous species, and show numerous polyploids is now difficult to say. Some possibilities to explain these phenomena will be perhaps evident from the results of the work on *Geum*. In any case it is evident that these plants are younger. Many of their numerous amphiploids grow on glaciated areas, and their origin was probably influenced by Pleistocene migrations and partly also in most recent times by man. Some of the relationships among different taxa of the tribe Geeae are shown in figure 1.

The Relationships Among the Species of the Genus *Geum*

1. Morphological differentiation

All Geeae are mesic herbaceous plants with more or less prominent caudex, numerous odd-pinnate, pinnatifid or lobed basal leaves, and more or less branched floral stems. They are normally outcrossed but self-compatible. The flowers are pentameric with a calyx tube usually with epicalyx, 5 sepals, 5 petals of different size and shape. Petals are usually yellow but sometimes white or red. Stamens as well as pistils are numerous, from 20 to 300 on an elongated receptacle. The most distinctive trait in these plants is the structure of the styles. It has been widely used as a diagnostic character in separating genera and species. The style is on the top of the ovary and is very elongated and persistent on ripe achenes in the tribes Cercocarpeae and Dryadeae as well as in some subgenera of *Geum*. It forms a long pinnately haired organ on the achenes well adapted to wind dispersal. In the subgenus Oncostylus from the Southern Hemisphere, the stigmatic part on the top of persisting styles is bent down and forms a hook adapted to dispersal by animals. In the biggest subgenus Eugeum the lower part of the style, called the rostrum, forms a hooked organ and the stigmatic part is shed after pollination. In the relic Mediterranean subgenus Orthurus the stigmatic part is shed but the rostrum is straight and forms on the top strong, down-pointing bristles perfectly adapted to dispersal by animals. In one species *G. speciosum* from the Caucasus (subg. Woronowia) nearly the whole style is shed after pollination.

In the two genera related to *Geum*, *Waldsteinia* and *Coluria*, the whole styles are dropped after pollination. The structure of the styles is of the greatest importance for seed dispersal and shows the biggest differentiation in the genus.

2. Geographical distribution

The genera of the tribes Cercocarpeae and Dryadeae except *Dryas* are restricted to Western North America. The genus *Dryas* with about 14 species has a wide distribution in the arctic regions and in many mountain ranges. The largest genus *Geum* has been divided into 11 subgenera (Gajewski, 1957) that have distinctive morphology and geographic distribution. The subgenera Sieversia and Neosieversia, with long pennate styles, grow in arctic or alpine zones of North-East Asia, Japan and Alaska. The subgenus Oreogeum with the similar structure of the styles is confined to the mountains of Europe. Another subgenus, Erythrocoma, with very long, pinnate, persistent styles, is North American chiefly in the Western U. S. with one species (*G. triflorum*) growing in Canada and along the Great Lakes to the Eastern Coast. The subgenus Acomastylis with the styles straight and persistent but much shortend and less hairy is confined chiefly to arctic and subarctic zones on both sides of Behring Strait (*G. Rossii, G. calthifolium*) but with species related to *G. Rossii* in the Rocky Mts. and to *G. calthifolium* in the White and Roan Mts. of the eastern U. S. Two species occur in the Himalaya Mts. In the Southern Hemisphere

LEVEL OF POLYPLOIDY	WALDSTEINIA	COLURIA	GEUM: OREOGEUM	GEUM: ACOMASTYLIS	GEUM: ERYTHROCOMA	GEUM: EUGEUM	GEUM: STYLIPUS	GEUM: ORTHURUS	GEUM: WORONOWIA	TOTAL NUMBER OF SPECIES
2n·84						G. magellanicum G. riojense G. pyrenaicum G. Fauriei				4
2n·70			G. bulgaricum			G. quellyon			G. speciosum	3
2n·56				G. Rossii G. turbinatum						2
2n·42	W. ternata		G. reptans	G. rotundifolium G. Peckii	G. triflorum G. ciliatum G. campanulatum	G. rivale G. coccineum G. canadense & 12 others	G. vernum			23
2n·28			G. montanum					G. heterocarpum		2
2n·14	W. geoides	C. geoides								2

FIG. 2. Chromosome numbers in some species of the genera *Geum, Waldsteinia* and *Coluria.*

in Tasmania, New Zealand, Auckland Islands and southernmost South America subgenus Oncostylus occurs with a hooked stigmatic part.

The biggest subgenus Eugeum (25 species) with jointed styles and hooked rostrum has the widest distribution, with several species in Europe, North America, and Asia but also along the Andes in South America and with one species in South Africa. The small subgenera, Orthurus (2 species) and Woronowia (1 species), are known only from the Mediterranean Region and from the Caucasus Mts. The related genera *Coluria* and *Waldsteinia,* with deciduous styles, are restricted to China (*Coluria*) and Atlantic America, East Asia and Europe (*Waldsteinia*). This kind of distribution suggests a past continuous distribution in arctotertiary forests.

3. Karyological relationships

Species in this group have rather small not clearly differentiated chromosomes from 1.5μ to 2.5μ long. As is evident from figure 2, the whole tribe has the basic chromosome number $x = 7$. Diploid species were found only in the genera *Waldsteinia* and *Coluria.* All *Geum* species known cytologically are polyploids. The more widespread condition is hexaploidy, which was found in most Eugeum species as well as in the subgenera Erythrocoma, Acomastylis, and Oreogeum. Dodecaploids with $2n = 84$ are known only from the subgenus Eugeum. Other polyploid levels like tetraploid, octoploid and decaploid are rare and scattered among different subgenera. Meiosis in pure species is usually quite normal with only a few rare univalents in some species. Multivalents were never observed, prob-

ably because of the low chiasma number at metaphase. In the subgenus Eugeum, which is the best known cytologically, no number of chromosomes lower than hexaploid were found. It seems that for this subgenus the triploid number 21 is a secondary basic number.

4. Crossability among different taxa

Many attempts to cross *Waldsteinia* and *Coluria* with different *Geum* species were completely unsuccessful, but in some combinations seeds with small embryos were obtained, which seems to indicate that viable hybrids could perhaps be obtained with the use of embryo culture. At present they represent separate comparia.

As figure 3 shows, at least one species from every subgenus tested could be crossed with one or more species of another subgenus or subgenera, with the exception of *G. Rossii* from the subgenus Acomastylis. However, other species of Acomastylis probably would form hybrids with other subgenera of *Geum*. Therefore, the entire genus probably forms one large comparium. Usually the ease in obtaining interspecific hybrids is much greater in intrasubgeneric crosses than in intersubgeneric ones. Some specific combinations between subgenera were always

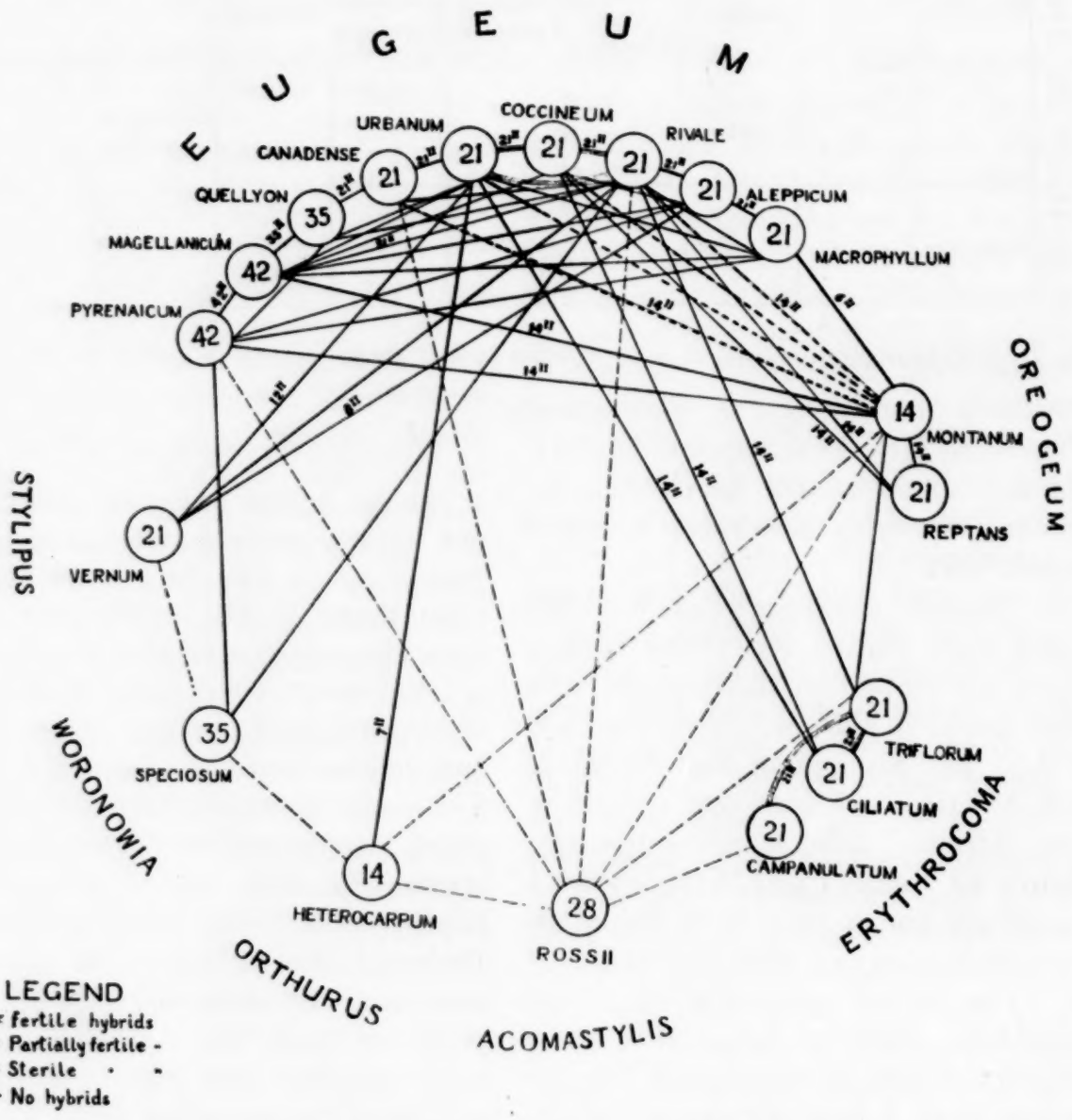

FIG. 3. Diagram representing the results of some inter- and intra-subgeneric crosses in the genus *Geum* L. The circles represent different species with their haploid chromosome numbers. The numbers along the lines connecting species indicate maximum of chromosome conjugations in F_1 hybrids.

a failure whereas other combinations give viable hybrids rather easily. In intrasubgeneric combinations the results were much better. Every interspecific pollination between Eugeum species has given viable hybrids, notwithstanding the differences in morphology, geographic distribution or chromosome numbers of the parental species. The same is true for the subgenera Erythrocoma and Oreogeum. Most of the F_1 hybrids are vigorous and flower abundantly. Some combinations, especially among intersubgeneric hybrids, are weak and difficult to grow at the seedling stage, but after the critical stage they usually develop into strong, luxuriant hybrids.

It may be concluded that within the subgenera a high level of crossability exists. The different subgenera are already partially separated from each other by incomplete barriers of incompatibility. One important exception must be pointed out here, namely that *G. montanum* from the subgenus Eugeum forms hybrids with species of the subgenus Eugeum with the greatest ease and the hybrids are partially fertile notwithstanding the great morphological differences in the structure of the styles and in the chromosome numbers of the parental species.

5. Hybrid fertility

As figure 3 shows, the hybrids between different subgenera are usually sterile. Here again very important exceptions are the hybrids between *G. montanum* and hexaploid Eugeum species which are partially fertile, with the seed fertility in some combinations ranging up to 34%. The hybrids between the species of the same subgenus may be completely sterile, partially fertile or even nearly completely fertile. Usually hybrids between species with different chromosome numbers are nearly sterile due to meiotic disturbances. The fertility of 55 studied interspecific hybrids among different hexaploid Eugeum species with at least potential complete chromosome conjugation at meiosis with 21 bivalents also

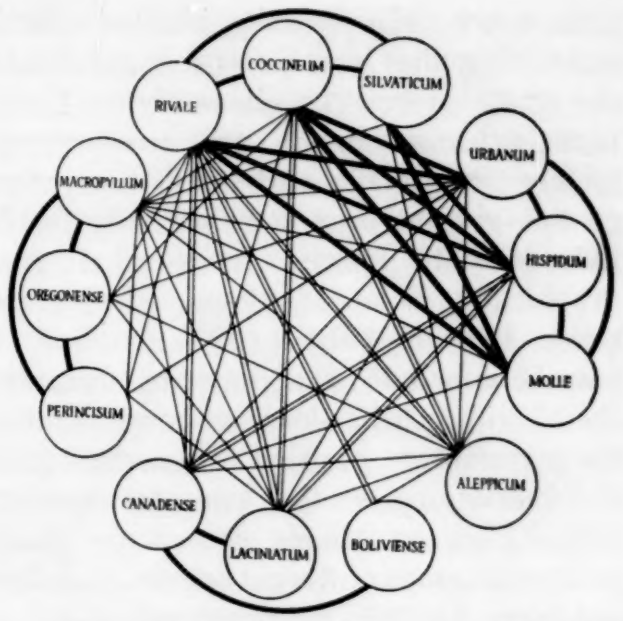

FIG. 4. Diagram illustrating hybrid fertility in F_1 hybrids among different hexaploid Eugeum species. The solid black lines indicate fertile hybrids, double lines—partially fertile hybrids, and single lines—sterile hybrids.

shows great variation according to the parental species used for the cross. It is clear that the fertility of the hybrids depends on specific parental gene combinations that are brought together in the hybrids. These genes have different effects on different hybrids: some prevent flowering altogether, others cause disturbances in the development of the floral parts, still others are genes causing asynapsis and last there are genes that cause breakdown of gametophyte development without affecting in a visible way the course of meiosis. In the subgenus Eugeum, the American species *G. macrophyllum* with two closely related species are nearly completely isolated from all other Eugeum species by hybrid sterility barriers caused by genes for asynapsis. The complicated fertility relationships in hybrids among 13 hexaploid Eugeum species are shown in figure 4. These species according to hybrid fertility could be arranged in five groups. The *rivale, coccineum, silvaticum* group gives fully or partially fertile hybrids with all other groups except with the *macrophyllum, oregonense, perincisum* group. This North American group is completely separated by hybrid sterility barriers from all Eugeum species. Other

groups are only partially isolated. It is interesting that the European species of the *rivale* group as well as of the European *urbanum* group are nearly completely interfertile, whereas the other groups give at least only partially fertile hybrids with species from other geographically isolated groups. From the many hybrids (about 150) studied, it may be concluded that in many instances the hybrid fertility does not correspond to the taxonomical position or morphological differentiation of the parental species. Sterility is sometimes caused by genes scattered among different species and does not have any phylogenetic meaning. It seems that in the subgenus Eugeum a very peculiar correlation does exist between the primitiveness of specific traits in the European species and their capacity to form fertile hybrids with other more advanced Eugeum species. More advanced species are more strongly separated from other species by hybrid sterility barriers.

6. Chromosome homology

As figure 3 shows, the chromosomes of the species from the different subgenera are only partially homologous. For instance hexaploid *G. triflorum* from the subgenus Erythrocoma has only two 7-chromosome genomes in common with hexaploid Eugeum species and only one with tetraploid *G. montanum* from the subgenus Oreogeum. Tetraploid *G. heterocarpum* (subg. Orthurus) has only one homologous genome with the hexaploid Eugeum species.

On the other hand, the many hybrids between hexaploid Eugeum species and the tetraploid *G. montanum* from the Oreogeum subgenus form 14 bivalents and 7 univalents at meiosis showing that all 14 *montanum* chromosomes are homologous with Eugeum chromosomes. The third genome of the hexaploid *G. reptans* from the Oreogeum subgenus is not homologous with the third genome of the subgenus Eugeum, and in the hybrids 14 bivalents and 14 univalents are formed. The other two genomes of this species are the same as in *G. montanum*.

Usually the hybrids between species with the same chromosome number and from the same subgenus show at least potential complete pairing with only bivalents present at meiosis. This is true for all hybrids between the hexaploid Eugeum or Erythrocoma species. In the hybrids between species with different chromosome numbers belonging to the same subgenus, as for instance in hybrids between hexaploid and decaploid or dodecaploid Eugeum species in meiosis, 21 bivalents are to be found and 14 or 21 univalents respectively.

The degrees of conjugation at metaphase I per PMC mentioned above are maximal ones. Usually in many PMC smaller numbers of bivalents and corresponding higher numbers of univalents are to be found. This is true also for the hybrids with potentially complete chromosome conjugation where PMC's with different numbers of univalents ranging from 2 to 10 are often to be found. The differences in the number of the univalents between different hybrids may be rather great.

In hybrids between *G. macrophyllum* and *G. aleppicum* on the one hand and other hexaploid Eugeum species on the other hand, complete asyndesis at metaphase I was found. In some instances plants with complete asynapsis and nearly normal pairing could be found at the same time. It seems that asynapsis at metaphase is rather caused by premature desynapsis. This in turn is primarily caused by some unknown internal and/or external conditions. The primary cause of desynapsis is clearly due to specific combinations of the genes brought together in the hybrid.

7. The inheritance of interspecific differences

Most of the traits of the interspecific F_1 are intermediate between those of the parental species, as for instance size and shape of the leaves, sepals and petals, kind

and degree of pubescence, time of flowering and many others. In F_2 populations of the fertile hybrids they show continuous variation, often transgressing the parental limits in one or both directions. Some characters like color or emargination of the petals or presence or absence of the anthocyanin in stems and floral parts are caused by one or only a few pairs of allelomorphic genes. The Mendelian segregation of these genes in F_2 or backcrosses of some fertile hybrids among hexaploid Eugeum species has been established. For instance *G. rivale* with cream-colored petals has the formula CCAAbb; *G. coccineum* with red petals has the formula CCaaBB. The F_1 hybrids with the formula CCAaBb are yellow and in the F_2 were found 169 plants with yellow petals, 62 with red petals and 68 with cream petals or nearly white which correspond fairly well to the theoretical ratio 9:3:4 (expected numbers 168.3: 56.1: 74.8). The factors A and B are both necessary for the production of the yellow pigment. All yellow flowering *Geum* species like *G. urbanum* for instance are of the formula CCAABB. The allele C is basic for any pigment formation and species with white petals like *G. canadense* were found to be of the ccAABB type. This was checked in many crosses in second generations and backcrosses involving different hexaploid Eugeum species. It seems likely that the same genes exist in the related subgenera Oreogeum and Erythrocoma but the evidence is not conclusive. The ratios found fit well with the expectations, which indicates that even in the partially fertile hybrids the gamete elimination in respect to these genes is not of a selective kind. There is no doubt that hexaploid Eugeum species even from different continents still possess homologous loci in their chromosomes. This may be true also for numerous genes with small cumulative effects but this is, of course, difficult to prove. From the analysis of many interspecific F_2 generations it is clear that species differ in very numerous genes with small effects affecting every part of the plant, but it is astonishing how quickly in backcrosses, after three or four backcross generations, the population is indistinguishable from recurring parental species. The influence of linkage of some of the specific traits in establishing parental types is very probable.

Very interesting is the inheritance of the style structure in the hybrids between *G. montanum* with straight, pennate styles and different Eugeum species with the hooked rostrum and deciduous stigmatic part. In the F_1 hybrids in the same individual, and even in the same flower, achenes develop with different types of styles, ranging from the straight to the hooked type. The structure of the styles is much influenced by age of the plant and time of flowering. In F_2 different types appear. Both parental types are segregated with a preponderance of the Eugeum type but also F_1 types with different styles in one plant appear. As the F_1 hybrids are pentaploid and only partially fertile, no clear ratios could be expected. In any case it is clear that the genes of the two parental species are coadapted to form one uniform type of structure of the styles, but when the genes from the two parental species are brought together in the hybrid their interaction is labile and is easily influenced by internal or external factors, and the degree of dominance and penetration of some of the genes is much changed or even sometimes reversed. The segregations in F_2 and backcross families seem to indicate that such a fundamental trait in the phylogeny of this group as the structure of the styles probably depends on few genes with major effects and many modifying genes.

The Evolution of the Genus *Geum*

From the results given above the author has traced the probable course of evolution of the group of plants investigated. The initial types were woody plants more or less related to the present genera of Dryadoideae now confined to

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Western North America. Probably during the Tertiary from plants such as these, herbaceous types have developed, for example, *Dryas* or the subgenera Sieversia and Neosieversia, now living mostly on unglaciated areas of the arctic regions of North-Eastern Asia and Alaska. As Hultén (1937) has shown, on these periglacial terrains many plants of arctotertiary type have survived the Pleistocene glaciations. These primitive Geeae all still had long-haired styles adapted to dispersal by wind. At the time when the mountains of the Alpine system were formed in Eurasia and America these plants migrated along the new mountain ranges far to the south and have after that formed more or less isolated colonies of mountain species like the subgenus Oreogeum in Europe, maintaining the primitive type of the styles. The Oreogeum species do not now show any close relations with arctic species, but the American mountain species from the subgenus Acomastylis still show close relations with arctic *G. Rossii* and *G. calthifolium*. At this time or somewhat later arose another American subgenus with straight pennate styles (Erythrocoma). This type of *Geum* with long hairy styles evolved mostly in the Northern Hemisphere and to a lesser extent in the Southern Hemisphere, where in the high mountains of Chile *G. andicolum* still has long, pennate styles as in northern subgenera. In the high mountains of Tasmania and New Zealand two species of the subgenus Oncostylus still have the same type of styles. Other species from the subgenus Oncostylus have later evolved much shorter and glabrous styles with a hooked stigmatic part that are well adapted to dispersal by animals. We know nearly nothing about the relationships between species from the Southern and Northern Hemispheres, but they seem to be related.

The northern subgenera Oreogeum, Erythrocoma and Acomastylis have only one or two common genomes. They have probably arisen by hybridization and amphiploidy between numerous largely differentiated diploid species, now completely extinct, and they present different combinations of their genomes. Within the same subgenus some species may have the same genome combinations and then the differences among them consist of numerous gene and small chromosome differences; others may represent different combinations of initial diploid species.

It is possible that at the same time as the mountain plants with persistent pennate styles were developed, or even earlier, there evolved in the forests along the mountains and on the plains, plants like modern genera *Coluria* and *Waldsteinia*. Their origin is quite obscure. They may have come from the primitive Geeae or even from other groups. They have styles that are shed by a special abcission layer formed at the base of the styles. Some species of *Coluria* have on the surface of the achenes thick papillae that are believed by Bolle (1933) to be an adaptation to dispersal by ants. Plants that shed their styles may have come in contact in many places along the mountains with plants that have persistent styles and may have hybridized. From such hybrids, plants could have arisen with long styles divided by an abcission layer in two parts: the lower persisting rostrum and the upper deciduous stigmatic part. From such hybrids could evolve new forms adapted to epizoochory by developing adhesive organs on the top of the rostrum.

The most successful type was the Eugeum type in which the tip of the rostrum was bent in a hook forming a perfect organ for adhering to animals. This group most probably arose as an amphiploid between tetraploid Oreogeum species and some other diploid species with deciduous styles as in *Waldsteinia*. The complete homology of the two genomes of the hexaploid Eugeum species with the two genomes of *G. montanum*, and the high fertility of the hybrids strongly suggest that they are nearly re-

lated. The structure of the stigmatic part in the Eugeum species is nearly the same in *Waldsteinia*. These facts support the supposition that the subgenus Eugeum has arisen as an amphiploid from hybrids between species related to *G. montanum* and diploid *Waldsteinia*. From the geographical distribution of parental species as well as from the fact that the most primitive species of Eugeum are centered in South Europe it seems probable that the subgenus Eugeum originated in mountains around the Mediterranean Basin. From this European center of origin this new subgenus well adapted to long distance transport by animals has shown tremendous migrations. Species of Eugeum have arrived in North America, then spread to Eastern Asia. From North America they have spread along the mountains to South America. One species is found even in the mountains of South Africa (*G. capense* in Natal). All these species have the new secondary basic number $x = 21$. In different parts of their large distributional area they have formed different separate species due to mutations and selection in ecologically and geographically separated populations. Even now widely separated species, for example, the South American *G. boliviense*, the Balcan *G. coccineum*, the North American *G. canadense*, and the East Asiatic *G. japonicum*, still have homologous chromosomes and homologous loci, and hybrids among them are at least partially fertile.

In the last stage of evolution of this subgenus, hybridization among different hexaploid species and doubling of the chromosome number in the hybrids was the cause of the formation of dodecaploid species with 84 chromosomes like *G. pyrenaicum* in Europe or *G. magellanicum* in South America. This process has twice occurred spontaneously in the present author's cultures of hybrids between hexaploid Eugeum species. One of these new amphiploids was highly fertile, very vigorous, and bred true through four generations (Gajewski, 1954).

The newest period of evolution in the genus *Geum* is caused by man introducing species from one continent to another and by creating new environments where species only partially separated by genetical barriers meet and hybridize. For example a colony of Spanish *G. hispidum* is now established on South-Western shores of Sweden and is expanding inland. Its plants seem to be a little different from the plants of Spain. In England on the other hand Marsden-Jones (1930) has shown that the introgression of *G. urbanum* into *G. rivale* is very common in some parts of this country.

We see thus that the evolutionary history of *Geum* has been long and very complicated. It consisted chiefly in change from the mode of dispersal by wind to dispersal by animals. This change was achieved in different ways in different parts of its area. The most successful were amphiploids of the subgenus Eugeum that unite genomes from very different plants. Once this amphiploid type was created it spread quickly and differentiated into many species chiefly by small gene and chromosome mutations. These species are often separated only by geographical or ecological barriers. Now they are again sufficiently different to form new amphiploids with the highest chromosome number, 84. They are still aggressive and enlarging their areas. The old remains of the primitive anemochoric type are now more restricted, some are relic and strictly monomorphic. They do not possess in general the aggressive properties of the younger Eugeum species. Some are doomed to complete extinction like the primitive diploid *Geum* species that seem to have disappeared completely a long time ago. We may guess that in the future only Eugeum species will actively evolve. The influence of man may initiate a new epoch of their rapid evolution.

Summary

Data on morphological characters, geographical distribution, chromosome num-

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bers, crossability between species, hybrid fertility, chromosome homology and inheritance of the interspecific differences of different species and hybrids in the genus *Geum* L. (Rosaceae) are presented and are used to trace the evolutionary history of the group studied. The results indicate that the genus *Geum* is a polyploid complex with many groups of species representing different evolutionary stages.

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Literature Cited

Bolle, F. 1933. Ein Uebersicht ueber die Gattung *Geum* L. und ihr nahestehenden Gattungen. Repert. Spec. Nov. Fedde, Beih. **72**: 1–119.

Gajewski, W. 1948. On the chromosome pairing in six hybrids among four *Geum* species. Acta Soc. Bot. Pol., **19**: 245–49.

——. 1949. On the behavior of univalents at meiosis in some interspecific *Geum* hybrids. Hereditas, **35**: 221–41.

——. 1950. The inheritance of specific traits in the hybrid *Geum coccineum* × *rivale*. Acta Soc. Bot. Pol., **20**: 489–516.

——. 1952. The hybrids between two subgenera of *Geum*. Acta Soc. Bot. Pol., **21**: 489–516.

——. 1953. A fertile amphipolyploid hybrid of *Geum rivale* with *G. macrophyllum*. Acta Soc. Bot. Pol., **22**: 411–39.

——. 1954. An amphiploid hybrid of *Geum urbanum* and *G. molle*. Acta Soc. Bot. Pol., **23**: 259–78.

——. 1955. The cytogenetic relationships among *Geum macrophyllum*, *G. perincisum*, and *G. oregonense*. Acta Soc. Bot. Pol., **24**: 319–34.

——. 1957. A cytogenetic study on the genus *Geum* L. Monogr. Bot., **4**: 1–414.

Hultén, E. Outline of the history of arctic and boreal biota during the Quaternary period. Stockholm, 168 pp.

Juzeptchuk, S. 1941. Rosoideae. Flora URSS, vol. X, Moscow.

Marsden-Jones, E. M. 1930. The genetics of *Geum intermedium* (Willd.) Ehrh. and its backcrosses. Jour. Genet., **22**: 377–95.

Rydberg, P. A. 1898. Some changes in the nomenclature of North American Rosaceae. Bulletin Torrey Bot. Club, **25**: 54–56.

——. 1908–1916. North American Flora, 22–24.

GENETICS OF NATURAL POPULATIONS. XXVIII. SUPPLEMENTARY DATA ON THE CHROMOSOMAL POLYMORPHISM IN *DROSOPHILA WILLISTONI* IN ITS RELATION TO THE ENVIRONMENT

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Introduction

Natural populations of *Drosophila willistoni* contain a greater variety of chromosomal polymorphs than those of any other species of Drosophila, or for that matter, of any other organism studied in this respect. As many as 50 different chromosomal inversions have been recorded. Populations which live in different parts of the distribution area are, however, by no means uniform. In Goias, in central Brazil, 34 different inversions have been found; the mean number of heterozygous inversions per individual (female) is about 9, and one individual was seen heterozygous for 16 inversions; individuals which are heterozygous for the same set of inversions are rather rare. By contrast, on the island of St. Kitts, in the Lesser Antilles, only 2 kinds of inversions have been recorded, the average number of heterozygous inversions per individual is 0.2, and most individuals are structural homozygotes. Intermediate situations have been found in other populations.

The question which unavoidably presents itself, is, what causes some populations to be so much more polymorphic than others? Da Cunha, Burla, and Dobzhansky (1950) advanced the working hypothesis that the chromosomal polymorphism is adaptive, and the populations which exploit a greater variety of ecological niches in the territory which they inhabit are more polymorphic than populations restricted to a narrow range of ecological opportunities. Further work on *D. willistoni* (Townsend, 1952; da Cunha and Dobzhansky, 1954; Dobzansky, 1957; Townsend 1958) disclosed new facts most of which were in agreement with the hypothesis, but some of which contradicted it. Thus, populations of the central part of the distribution area are more polymorphic than geographically marginal populations; populations of territories with a greater diversity of habitats suitable for the fly to live in are more polymorphic than those of more uniform or of unfavorable territories; the populations are more polymorphic where *D. willistoni* is the sole representative of its species group than where it shares the habitats with its close relatives, and especially where the latter are more common and presumably more successful; and populations of continental masses are more polymorphic than those of oceanic islands or of distributional pockets. The contradictory observation has been that the exuberant forests on the coasts of the states of Bahia and Espirito Santo, in Brazil, where *D. willistoni* is the dominant species of its group, are inhabited by populations which show almost as little polymorphism as those of the adjacent desert regions.

The purpose of the work reported in the pages that follow has been twofold. First, we attempted to obtain and to study population samples of *D. willistoni* from those parts of the geographic area of the species which remained unknown. Secondly, we made special efforts to study populations in territories for which, on the basis of our working hypothesis,

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predictions could be formulated regarding the extent of the chromosomal polymorphism to be expected in them. The validity of a working hypothesis is, after all, tested by its ability to predict the situations that can be observed in materials collected for that purpose.

Material

Drosophila willistoni inhabits a territory extending from southern Florida and central Mexico to the La Plata and the eastern foothills of the Andes near Tucuman, in Argentina. From this enormous distribution area, data are available on the composition of population samples of varying degrees of adequacy from 78 localities, as shown on the maps in figures 1 and 2. The locality numbers on these maps correspond to those in tables 1–5, but the maps show also the localities for which the data have been published previously (da Cunha, Burla, and Dobzhansky, 1950; Townsend, 1952; da Cunha and Dobzhansky, 1954; Dobzhansky, 1957). All the data in tables 1–4 are new except for the inclusion of the figures given by Townsend (1958) for Puerto Rico (No. 6A) and for Trinidad (No. 21A).

The new collections have been made in the following localities. 6B—Mayaguez and El Yunque, Puerto Rico, October 1957, M. Wasserman; 7—St. Thomas, B.W.I., August 1957, W. Heed; 10—St. Vincent, Mesopotamia Valley, B.W.I., July 1957, W. Heed; 12—Tikal, Guatemala, May 1958, S. D. Coe; 16—Panama, February 1958, M. R. Wheeler; 17—Monteria, Colombia, February 26, 1958, D (= Th. Dobzhansky, Collector); 20—Caripe, Venezuela, November 1956, M. Wasserman; 21B—near Port of Spain, Trinidad, July 1957, W. Heed; 21C—Tabaquite, Trinidad, July 1957, W. Heed; 22—east of Buenaventura, and the valley of the Anchicaya River, Colombia, March 6, 1958, D; 23—Palmira, Colombia, March 7, 1958, D; 24—midway between Villavicencio and San Martin, Llanos, Colombia, March 19, 1958, D and Lazzaro Posada; 25—Santo Domingo de Los Colorados, Ecuador, March 14, 1958, D and Gerardo Naranjo; 26—Pichilingue, Ecuador,

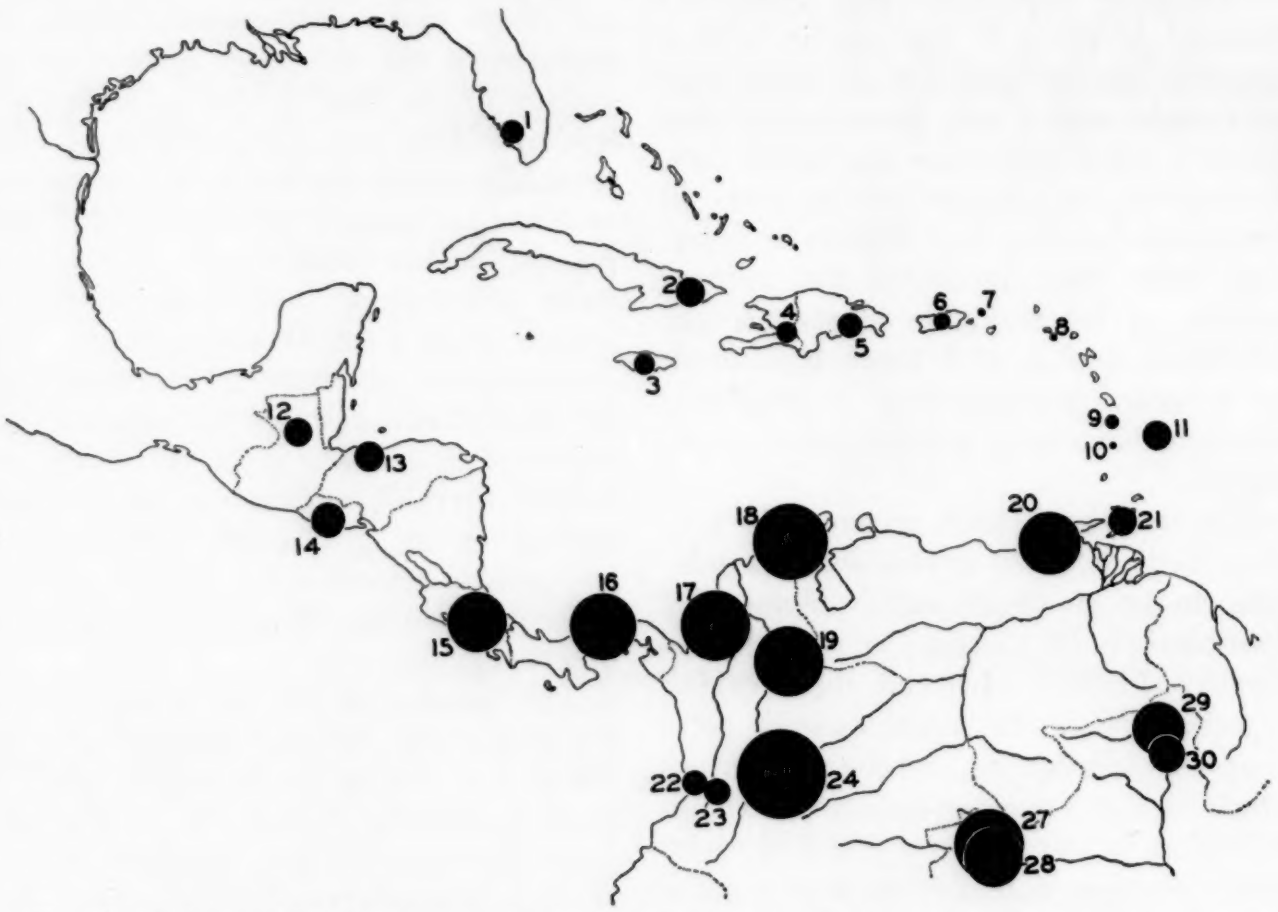

Fig. 1. Polymorphism in the populations of *Drosophila willistoni* in the northern part of the distribution area of the species. The diameters of the black circles are proportional to the mean numbers of heterozygous inversions per individual female. The numbers of the localities correspond to those in tables 1–5.

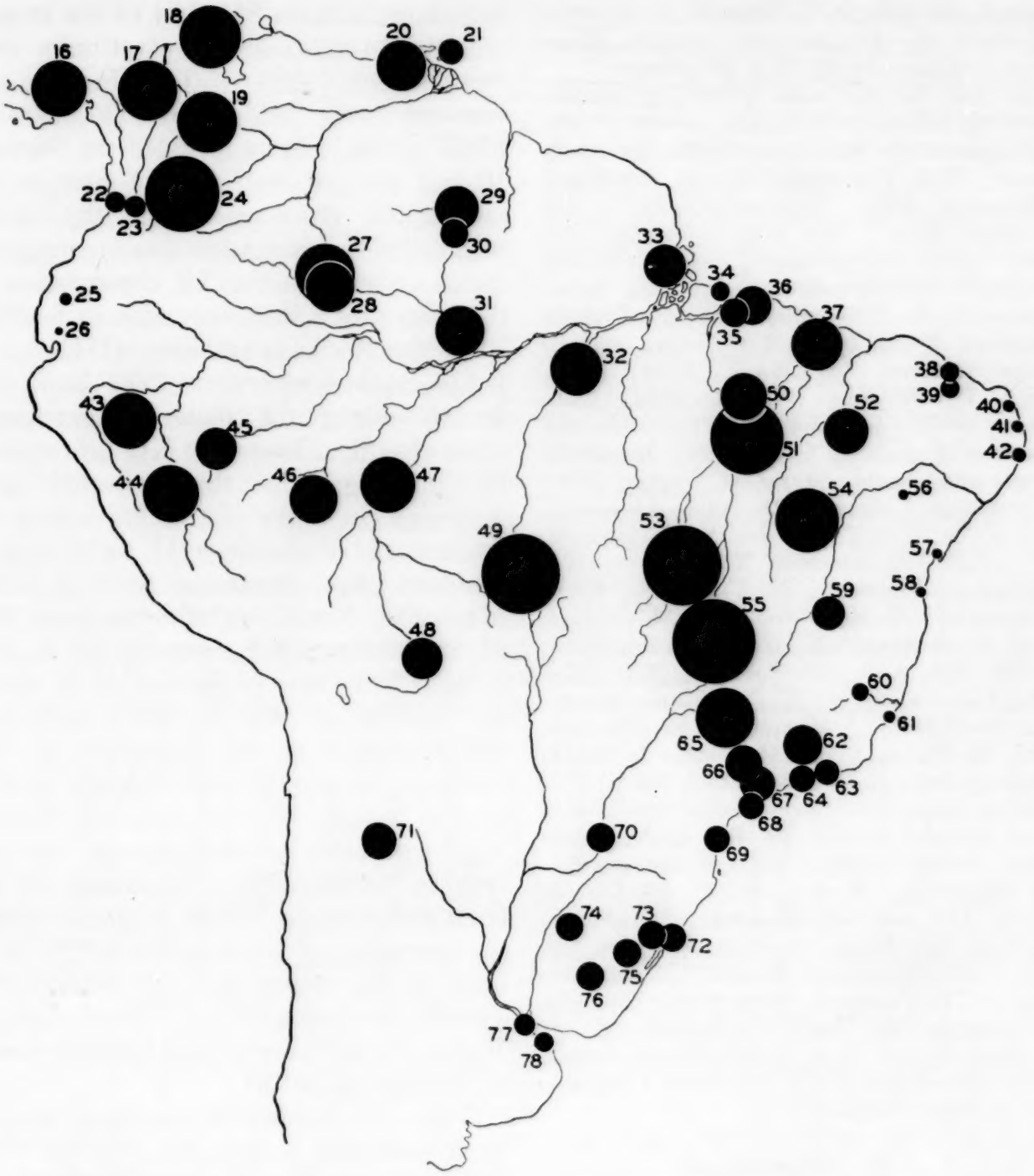

FIG. 2. Polymorphism in South American populations of *Drosophila willistoni*. The diameters of the black circles are proportional to the mean numbers of heterozygous inversions per individual female. The numbers of the localities correspond to those in tables 1–5.

March 10, 1958, D and M. Wheeler; 37A—Fazenda Salamanca, near São Luiz, Maranhão, Brazil, April 12, 1956, D and A.G.L. Cavalcanti; 37B—Rosario, Maranhão, April 13, 1956, D and A.G.L. Cavalcanti; 38—Serra Maranguape, south of Fortaleza, Ceara, Brazil, April 16–17, 1956, D and A.G.L. Cavalcanti; 39A—Guaramiranga, Serra Baturite, Ceara, Brazil, April 20, 1956, D and A.G.L. Cavalcanti; 39B—Aratuba, Serra Baturite, April 19, 1956, D and A.G.L. Cavalcanti; 40—Natal, Rio Grande do Norte, Brazil, April 22, 1956, D and A.G.L. Cavalcanti; 41—Santa Rita, near João Pessoa, Paraiba, Brazil, November 16–17, 1955, D and A.G.L. Cavalcanti; 42A—Olinda, Pernambuco, Brazil, November 13, 1955, D and A.G.L. Cavalcanti; 42B—Dois Irmãos, Recife, Pernambuco, November 12, 1955, D and A.G.L. Cavalcanti; 42C—as above, April 24, 1956, D, Ph. l'Heritier, and A.G.L. Cavalcanti; 43—Tarapoto, Peru, September 28, 1955, D and C. Pavan; 44—Tingo Maria, Peru, September 25–26, 1955, D and C. Pavan; 48—Santa Cruz, Bolivia, March 1958, M. Wasserman; 49—Seringal Divisão, Rio Verde, Mato Grosso, Brazil, August 19, 1955, D and Ch. Birch; 64—several collections on islands in the Bay of Angra dos Reis, State of Rio de Janeiro, Brazil,

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June and July 1955, D. C. Pavan, A. B. da Cunha and others; 65—Araguari and Ituiutaba, Minas Gerais, February 16–20, 1956, M. Breuer.

The data for the other population samples shown on the maps in figures 1 and 2 have been reported by da Cunha, Burla, and Dobzhansky, 1950; Townsend, 1952; da Cunha and Dobzhansky 1954; Dobzhansky, 1957; and Townsend, 1958. Very brief indications will suffice here, as follows. 1—Lake Placid, Florida; 2—Cienfuegos and Santiago, Cuba; 3—Jamaica; 4—Petionville, Haiti; 5—Trujillo, Dominican Republic; 6—Rio Piedras and El Yunque, Puerto Rico; 8—St. Kitts; 9—St. Lucia; 11—Barbados; 13—Lancetilla, Honduras; 14—El Salvador; 15—Turrialba, La Lola, and San Jose, Costa Rica; 18—Sierra Nevada de Santa Marta, Colombia; 19—Bucaramanga, Colombia; 27—Içana, Amazonas; 28—Foz de Caiari, Amazonas; 29—Savanna, Rio Branco; 30—Mucajai, Rio Branco; 31—Rio Negro, Amazonas; 32—Fordlandia, Para; 33—Ferreira Gomes, Amapa; 34—Marajo, Para; 35—Belem, Para; 36—Castanhal, Para; 45—Rio Moa, Acre; 46—Palmares, Acre; 47—Porto Velho, Guapore; 50—Imperatriz, Maranhão; 51—Carolina, Maranhão; 52—Floriano, Piaui; 53—Palma, Goias; 54—Barreiras, Bahia; 55—Monjolinho, Goias; 56—Catuni, Bahia; 57—Salvador, Bahia; 58—Ilheus, Bahia; 59—Montes Claros, Minas Gerais; 60—Rio Doce, Minas Gerais; 61—Sooretama, Espirito Santo; 62—Boa Esperança, Minas Gerais; 63—Rio de Janeiro, D.F.; 66—Pirassununga, São Paulo; 67—Mogi, São Paulo; 68—Vila Atlantica, São Paulo; 69—Paranagua, Parana; 70—Iguassu, Parana; 71—Tucuman, Argentina; 72—Feliz, Rio Grande do Sul; 73—Reuter, R.G.S. 74—Santo Angelo, R. G. S.; 75—Ponta Grossa, R.G.S.; 76—Bage, R.G.S. and Melo, Uruguay; 78—La Plata, Argentina.

The Inversions

Da Cunha, Burla, and Dobzhansky (1950) have published descriptions of 40 inverted sections found in heterozygous condition in the chromosomes of Brazilian populations of *D. willistoni*. The drawings of most of these inversions were also published in the paper just referred to, while a standard map of the giant polytene chromosomes of *D. willistoni* was given by Dobzhansky (1950). Townsend (1952) added the inversion III-A-1 in the third chromosome, which extends from the middle of Section 87 to the distal end of Section 92 of the standard map. The inversion N in the same chromosome, including Sections 81 to 84 of the standard map, was added by da Cunha and Dobzhansky (1954). Two further inversions were added by Dobzhansky (1957); one of them included the Section 10 and the proximal part of Section 11 of the XL chromosome (in the paper referred to this inversion was erroneously stated as being in the XR chromosome); the other was a short inversion in Section 94 of the third chromosome (III).

Six further inversions have been observed among the materials examined more recently. Inversion XL-J, first seen in the progeny of flies collected near Villavicencio, Colombia, includes most of the material in Sections 13 to 15 of the standard map. Inversion XR-C-1, from Maranhão, Brazil, includes Sections 20–25. Inversion XR-F, from Recife, Brazil, extends from base of Section 32 to about the middle of Section 35. Inversion IIR-F begins at the boundary of the Sections 56 and 57 and extends to the base of Section 70 (definitely not including the probably heterochromatic "repeat" area in Section 70). Inversion III-P, from Pernambuco, Brazil, begins at about the boundary of Sections 84 and 85 and ends in the distal part of Section 90. Finally, inversion III-V, from Caripe, Venezuela, includes a short block of genes in Section 80 and 81.

Thus, the number of inversions known in *D. willistoni* is now 50, distributed as follows in the five chromosome limbs:

XL	XR	IIL	IIR	III
11	7	8	6	18

Inversions are, accordingly, present in all the chromosomes, although their numbers may not be strictly proportional to the relative lengths of the chromosomes. Chromosome III is the longest and has most inversions; the second largest number of known inversions is, however, in the shortest chromosome, XL; and the smallest number of the inversions is in the second longest chromosome, IIR. Whether these discrepancies are statistically significant may, however, be ques-

tioned. There is also no obvious preference for "natural" inversions to occur in any particular part of any chromosome, as there is, for example, in *D. pseudoobscura* in which only two of the more than twenty inversions known in the third chromosome have one of the two breaks in the proximal one-third of the chromosome. In *D. willistoni,* four of the five chromosome limbs have each an inversion close to the base (except III), and four of the five have subterminal inversions (except XR). It looked as though the proximal part of the chromosome III was free of inversions, but the inversion III-V has removed this suspicion. The chromosomes in the salivary gland cells of *D. willistoni* are not good enough to facilitate precise localization of the breaks in the inversions. It may, however, be stated that coincidence of some breaks in two or more inversions at the same point in the chromosome is rare, if it occurs at all.

Some of the inversions occur almost everywhere in the species area. One of these is III-J (which has not, however, been recorded in the Buenaventura and Pichilingue samples, table 2). Others are IIL-E, IIL-F, and IIR-E. Such species-wide "universality" of polymorphs is characteristic of species of Drosophila associated with man, but it is unusual in widely distributed wild species. The "universal" polymorphs have evidently been able, impelled by natural

TABLE 1. *Frequencies (in per cent) of inversion heterozygotes in certain populations of* Drosophila willistoni

Inversion \ Locality	6A. Puerto Rico	6B. Puerto Rico	7. St. Thomas	10. St. Vincent	25. Santo Domingo, Ecuador	26. Pichilingue, Ecuador	38. Maranguape Ceara	39A. Guaramiranga, Ceara	39B. Aratuba, Ceara	40. Natal	41. João-Pessoa, Paraíba	42A. Olinda, Pernambuco	42B. Recife (1955)	42C. Recife (1956)
Chromosome XL														
None	98.3	100.0	100.0	100.0	100.0	96.0	100.0	98.4	94.6	98.7	100.0	100.0	97.8	97.2
A	1.7	—	—	—	—	4.0	—	—	—	—	—	—	—	—
F	—	—	—	—	—	—	—	—	5.4	—	—	—	—	—
H	—	—	—	—	—	—	—	1.6	—	1.3	—	—	2.2	2.8
Chromosome XR														
None	100.0	100.0	100.0	100.0	100.0	96.0	100.0	100.0	100.0	100.0	95.1	100.0	97.2	100.0
C + D	—	—	—	—	—	4.0	—	—	—	—	2.4	—	—	—
F	—	—	—	—	—	—	—	—	—	—	2.4	—	2.8	—
Females Studied	59	31	12	15	12	25	24	63	37	78	62	41	71	
Chromosome IIL														
None	56.0	64.9	85.7	100.0	66.7	87.5	89.2	96.7	92.6	93.9	97.3	95.5	91.7	91.7
A or B	43.0	17.5	7.1	—	8.3	6.2	2.7	1.1	—	1.5	—	1.5	4.9	4.9
A + B	—	—	—	—	—	3.1	2.7	2.2	2.9	0.8	—	—	—	—
D or E	8.0	5.3	—	—	8.3	—	2.7	—	1.5	0.8	—	—	2.4	2.5
D + E	—	—	—	—	—	—	—	—	—	0.8	—	—	—	—
F	—	12.3	7.1	—	33.1	3.1	2.7	—	2.9	1.5	2.7	3.0	3.6	1.6
G	—	—	—	—	—	—	—	—	—	1.5	—	—	—	—
Chromosome IIR														
None	57.0	63.2	71.4	100.0	88.3	90.6	37.8	35.2	55.9	76.9	78.2	79.1	78.6	72.1
A	18.0	—	—	—	—	9.4	—	—	2.9	2.3	8.8	7.5	8.3	9.0
B	—	—	7.1	—	—	—	—	—	—	—	—	—	1.2	2.5
E	30.0	35.1	21.4	—	—	—	62.2	64.8	41.2	20.8	15.4	13.4	11.9	16.4
F	—	1.8	—	—	8.3	—	—	—	—	—	—	—	—	—
Chromosome III														
None	73.0	68.4	100.0	73.3	66.7	93.8	16.2	11.0	20.6	10.2	23.6	22.4	29.7	18.0
B	1.0	—	—	6.7	16.6	3.1	—	4.4	—	—	—	—	1.2	—
C or E	—	—	—	—	—	3.1	—	—	—	—	—	—	—	—
D	—	—	—	—	—	—	2.7	—	—	2.3	—	—	—	—
F	—	—	—	—	—	—	—	—	—	—	0.9	—	1.2	0.8
H	—	—	—	—	8.3	—	16.2	29.7	33.8	14.6	15.4	5.9	7.1	16.8
J	26.0	31.6	—	13.3	16.6	—	64.9	54.9	48.5	60.8	63.6	65.7	61.9	60.7
L	—	—	—	6.7	8.3	—	—	—	—	—	—	—	—	—
P	—	—	—	—	—	—	—	—	—	16.9	18.2	26.9	27.4	36.1
Flies studies	100	57	12	15	12	32	37	91	68	130	110	67	84	122

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TABLE 2. *Frequencies (in per cent) of inversion heterozygotes in certain populations of* Drosophila willistoni

Inversion \ Locality	12. Tikal Guatemala	16. Panama	17. Monteria Colombia	20. Caripe Venezuela	21A. Trinidad	21B. Trinidad	21C. Trinidad	22. Buenaventura Colombia	23. Palmira Colombia	24. Villavicencio Colombia	37A. Sao Luiz Maranhao	37B. Rosario Maranhao	43. Tarapoto Peru	44. Tingo Maria Peru	48. Santa Cruz Bolivia	49. Rio Verde Mato Grosso	64. Angra dos Reis Rio de Janeiro	65. Araguari Minas Gerais
Chromosome XL																		
None	83.6	52.9	50.0	55.7	100.0	77.7	92.2	73.7	76.8	28.2	57.5	50.0	55.5	37.2	38.5	14.0	42.3	25.2
A	3.6	—	10.0	8.2	—	5.6	2.0	5.3	7.2	14.6	7.5	7.9	18.5	11.8	23.1	27.0	7.6	3.4
B	—	—	—	4.9	—	—	—	—	—	29.1	11.2	26.3	—	—	—	—	—	—
D	7.3	—	10.0	—	—	—	—	5.3	—	—	5.0	5.3	—	6.7	10.3	—	—	23.8
F	—	—	—	1.6	—	—	—	—	—	—	6.2	15.5	7.4	16.9	10.3	44.0	0.3	13.6
H	—	—	—	1.6	—	—	—	—	—	—	2.5	—	14.8	8.5	—	2.0	53.1	49.7
J	—	—	—	—	—	—	—	5.3	—	20.4	—	—	—	—	—	—	—	—
D + H	—	—	—	3.3	—	—	—	—	14.5	16.5	—	—	3.7	16.9	2.6	1.0	2.0	—
C + D	5.5	23.5	10.0	—	—	5.6	—	5.3	—	13.6	—	—	—	—	—	—	—	—
D + F + G	—	17.6	20.0	6.6	—	5.6	5.9	5.3	—	19.4	21.3	21.1	—	—	—	7.0	—	4.1
E + F + G	—	5.9	—	23.0	—	—	—	—	4.3	—	6.2	2.6	3.7	22.0	30.8	12.0	—	2.0
Chromosome XR																		
None	74.6	17.6	10.0	42.6	100.0	61.1	70.6	100.0	84.1	38.8	40.0	26.3	59.2	66.1	92.3	26.0	32.7	27.9
A	1.8	29.4	20.0	18.0	—	11.2	17.6	—	7.2	19.4	8.7	10.5	11.1	16.9	—	58.0	9.0	4.8
B	—	—	10.0	3.3	—	—	—	—	—	1.0	6.2	5.3	3.7	—	—	{69.0}	—	—
C	3.6	11.8	10.0	26.6	—	5.6	—	—	—	4.9	32.5	42.1	3.7	1.6	2.6		—	19.7
D	3.6	—	20.0	19.7	—	16.7	5.9	—	4.3	6.8	33.7	28.9	25.9	11.8	5.1	25.0	50.1	52.4
E	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	8.5	17.0
M	—	—	—	—	—	—	—	—	—	—	2.5	7.9	—	—	—	—	—	—
C + D	16.4	17.6	60.0	3.3	—	11.2	5.9	—	7.2	36.9	—	—	—	—	—	—	—	—
D + E	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3.8	—
Females studied	56	17	10	61	12	18	51	19	69	103	80	38	27	59	39	100	343	147
Chromosome IIL																		
None	60.0	17.4	25.0	19.5	73.7	40.0	47.4	59.1	53.4	7.4	19.0	14.3	4.5	4.2	23.4	1.5	17.0	24.3
A or B	21.8	8.7	41.7	25.3	4.8	20.0	10.5	22.7	26.0	17.2	14.0	15.9	40.9	52.6	48.9	36.0	17.9	22.0
A + B	—	26.1	25.0	17.2	—	16.7	7.0	9.1	2.7	45.9	47.0	52.4	31.8	17.8	10.6	46.3	—	2.7
C	—	—	—	—	—	—	—	—	—	—	—	—	—	1.0	—	1.5	1.5	—

TABLE 2. *Frequencies (in per cent) of inversion heterozygotes in certain populations of* Drosophila willistoni—(*Continued*)

Inversion \ Locality	12. Tikal Guatemala	16. Panama	17. Monteria Colombia	20. Caripe Venezuela	21A. Trinidad	21B. Trinidad	21C. Trinidad	22. Buenaventura Colombia	23. Palmira Colombia	24. Villavicencio Colombia	37A. Sao Luiz Maranhao	37B. Rosario Maranhao	43. Tarapoto Peru	44. Tingo Maria Peru	48. Santa Cruz Bolivia	49. Rio Verde Mato Grosso	64. Angra dos Reis Rio de Janeiro	65. Araguari Minas Gerais
D or E	21.8	21.7	16.7	33.3	19.0	10.0	15.8	4.5	5.5	26.2	16.0	11.1	52.2	27.3	19.1	58.2	56.3	41.3
D + E	—	8.7	16.7	14.9	—	10.0	12.3	—	1.4	11.5	2.0	4.8	—	—	—	—	1.9	1.2
F	3.6	34.8	16.7	49.4	—	20.0	17.5	27.3	31.5	68.9	36.0	39.7	56.8	64.2	44.7	50.7	55.1	53.3
G	—	4.3	—	—	—	—	—	—	—	—	—	—	—	—	2.1	—	—	—
H	—	—	—	1.1	—	6.7	—	—	—	8.2	3.0	1.6	27.2	22.1	2.1	20.6	1.7	7.3
Chromosome IIR																		
None	41.8	30.4	25.0	48.3	68.4	60.0	71.9	54.5	76.7	45.9	78.0	69.8	34.0	34.7	59.6	48.5	92.0	58.7
A	14.5	26.1	50.0	13.8	—	3.3	14.0	18.2	—	18.0	10.0	20.6	34.0	26.3	8.5	27.9	2.4	0.4
B	—	—	—	1.1	—	—	1.8	—	1.4	5.7	1.0	1.6	2.2	—	—	8.1	—	—
C	—	8.7	—	1.1	—	10.0	—	—	—	—	2.0	—	—	—	—	6.6	0.7	1.5
D	—	—	8.3	—	—	—	—	—	—	—	—	—	—	—	—	0.7	—	—
E	56.4	47.8	41.7	41.4	31.6	26.7	12.5	36.4	8.2	36.9	9.0	7.9	43.2	49.4	34.0	16.2	4.7	40.2
F	—	—	—	—	—	—	—	—	13.7	2.5	—	—	—	—	—	—	—	—
Chromosome III																		
None	58.2	26.1	8.3	26.4	26.3	40.0	47.4	59.1	74.0	9.0	24.0	28.6	9.1	5.2	21.3	5.9	34.6	8.5
A − 1	—	—	—	3.4	—	—	—	—	—	—	—	—	—	—	—	—	—	—
A or B	—	4.3	—	24.1	—	—	8.8	22.7	4.1	31.1	6.0	1.6	54.5	49.4	31.9	44.9	44.5	36.7
A + B	—	8.7	8.3	2.3	—	—	—	9.1	—	22.1	6.0	3.2	13.6	23.1	4.3	23.5	0.2	5.0
C or E	—	4.3	—	—	—	—	3.5	4.5	—	18.0	2.0	—	2.2	1.0	—	3.7	—	26.3
D	—	17.4	25.0	18.4	—	—	—	—	—	24.6	19.0	19.0	15.9	26.3	12.8	26.5	0.2	10.4
D + E	—	—	—	—	—	—	—	—	—	11.0	12.7	—	—	—	—	—	—	—
F	—	34.8	16.7	31.0	—	6.7	—	27.3	—	26.2	18.0	31.7	—	2.1	14.9	37.5	0.2	40.2
H	—	13.0	8.3	24.1	—	10.0	—	—	13.7	54.9	12.0	12.7	45.4	48.4	36.2	58.1	0.2	48.3
I	—	—	—	—	—	—	—	—	—	1.6	—	—	4.5	2.1	2.1	2.9	0.2	0.4
J	41.8	13.0	66.7	19.5	47.4	20.0	14.0	—	5.5	15.6	53.0	47.6	15.7	13.6	10.6	8.1	44.2	15.8
L	—	30.4	8.3	14.9	15.8	30.0	14.0	—	4.1	33.6	—	4.8	38.6	42.1	36.2	54.4	—	0.4
L + M	—	4.3	—	2.3	10.6	6.7	19.3	—	—	12.3	—	—	2.2	6.3	—	14.7	—	49.0
U	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2.9	—	—
V	—	—	—	6.9	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Flies studied	55	23	12	87	19	30	57	22	73	122	100	63	44	95	47	136	535	259

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selection, to diffuse to all parts of the species area from wherever they arose originally. For a species distributed over a territory as large as *D. willistoni* this means a great deal of migration. On the other hand, some inversions are endemics restricted to only a small geographic region. These polymorphs may be relatively new, or they may carry gene complexes adaptive only in a certain region.

As stated in the Introduction, the degree of the polymorphism is quite variable in different places. Inspection of figures 1 and 2 will show that the zone of highly polymorphic populations (large circles) occupies the interior of the South American continent. It extends from western Bahia (No. 54), through central Brazil, almost the whole Amazon Basin, to the eastern slopes of the Andes in Peru and in Colombia, and to the northeastern corner of the continent (Nos. 17–19, fig. 2). The polymorphism becomes steadily reduced as one moves towards the southern distribution limits, in southern Brazil, Uruguay, and Argentina. It is also reduced towards the northern distribution margins, in Central America, West Indies, and especially in the Lesser Antilles. Another sharp reduction is observed in northeastern Brazil, a zone of deserts and other submarginal habitats.

Correlation Between Polymorphism and Environment

Using a suggestion of Dansereau (1952), da Cunha and Dobzhansky (1954) have attempted to evaluate, in

Table 3. *Mean members of heterozygous inversions per individual in different populations*

Locality	Females	Males
6A. Puerto Rico	1.24 ± 0.12	1.34 ± 0.13
6B. Puerto Rico	1.16 ± 0.16	0.84 ± 0.14
7. St. Thomas	0.50 ± 0.19	—
10. St. Vincent	0.27 ± 0.11	—
12. Tikal, Guatemala	2.22 ± 0.18	—
16. Panama	6.18 ± 0.51	—
17. Monteria, Colombia	6.70 ± 0.75	—
20. Caripe, Venezuela	5.79 ± 0.34	3.62 ± 0.34
21A. Trinidad	1.50 ± 0.15	1.14 ± 0.40
21B. Trinidad	3.18 ± 0.29	2.58 ± 0.29
21C. Trinidad	2.33 ± 0.20	—
22. Buenaventura, Colombia	2.11 ± 0.38	—
23. Palmira, Colombia	2.23 ± 0.18	—
24. Villavicencio, Colombia	8.65 ± 0.21	4.79 ± 0.40
25. Santo Domingo, Ecuador	1.17 ± 0.38	—
26. Pichilingue, Ecuador	0.44 ± 0.13	0.14 ± 0.16
37A. São Luiz, Maranhão	5.58 ± 0.25	4.05 ± 0.32
37B. Rosario, Maranhão	6.05 ± 0.36	4.56 ± 0.33
38. Maranguape, Ceara	1.79 ± 0.23	1.31 ± 0.28
39A. Guaramiranga, Ceara	1.76 ± 0.15	1.29 ± 0.20
39B. Aratuba, Ceara	1.41 ± 0.17	1.48 ± 0.18
40. Natal, Rio Grande Norte	1.19 ± 0.09	1.29 ± 0.10
41. João-Pessoa, Paraiba	1.20 ± 0.11	1.25 ± 0.13
42A. Olinda, Pernambuco	1.17 ± 0.12	1.46 ± 0.15
42B. Recife (1955), Pernambuco	1.37 ± 0.15	1.29 ± 0.17
42C. Recife (1956), Pernambuco	1.58 ± 0.09	1.29 ± 0.11
43. Tarapoto, Peru	6.18 ± 0.28	5.23 ± 0.40
44. Tingo Maria, Peru	6.52 ± 0.26	5.39 ± 0.26
48. Santa Cruz, Bolivia	4.72 ± 0.32	—
49. Rio Verde, Mato Grosso	9.15 ± 0.25	6.22 ± 0.23
64. Angra dos Reis, Rio de Janeiro	3.52 ± 0.10	2.29 ± 0.09
65. Araguari, Minas Gerais	7.12 ± 0.20	4.49 ± 0.21

TABLE 4. *Observed proportions (in per cent) of the four sibling species in the samples examined*

Sample	*willistoni*	*equinoxialis*	*tropicalis*	*paulistorum*	Flies examined
6B. Puerto Rico	46.1	44.9	9.0	—	167
7. St. Thomas	94.1	5.9	—	—	17
10. St. Vincent	100.0	—	—	—	9
12. Tikal	95.1	3.1	1.5	—	65
16. Panama	47.1	41.1	9.8	1.9	51
17. Monteria	8.2	3.1	88.7	—	159
20. Caripe	72.1	5.9	6.6	15.4	136
21B. Trinidad	62.5	31.2	—	6.3	48
21C. Trinidad	47.3	22.3	1.4	29.1	148
22. Buenaventura	51.3	—	—	48.7	41
23. Palmira	88.0	—	6.7	5.3	75
24. Villavicencio	73.1	14.1	10.3	2.6	156
25. Santo Domingo	72.3	—	22.2	5.6	17
26. Pichilingue	58.2	—	38.2	3.6	55
37A. São Luiz	74.1	—	0.7	25.2	135
37B. Rosario	55.8	—	1.8	42.5	113
38. Maranguape	45.6	—	3.7	50.6	81
39A. Guaramiranga	95.8	—	1.1	3.1	96
39B. Aratuba	95.8	—	1.4	2.8	71
40. Natal	100.0	—	—	—	130
41. João-Pessoa	97.3	—	0.9	1.8	113
42A. Olinda	95.7	—	—	4.3	70
42B. Recife (1955)	89.4	—	—	10.6	94
42C. Recife (1956)	76.2	—	0.6	23.1	100
43. Tarapoto	56.2	3.8	2.5	37.5	80
44. Tingo Maria	67.8	0.7	2.9	28.6	140
49. Mato Grosso	100.0	—	—	—	136
64. Angra dos Reis	83.3	—	—	16.7	642
65. Araguari	89.0	—	0.3	10.7	291

quasi-quantitative terms, the characteristics of the environments in different parts of Brazil, which might be expected, on the basis of our working hypothesis, to favor greater or lesser polymorphism in populations of *D. willistoni*. The environmental ratings thus obtained were then correlated with average numbers of the inversions for which an individual is heterozygous in different territories. A positive correlation has been observed. However, three populations (Nos. 58, 60 and 61 in fig. 2, inhabiting the coastal forests) have been found to be conspicuously less polymorphic than the environmental ratings led us to expect.

The most highly polymorphic populations are those of Goias (Nos. 53 and 55 in fig. 2), with 9.11 ± 0.44 and 9.36 ± 0.26 inversions per individual (female) respectively. Their environmental ratings are 12 and 11, as shown in table 4 in da Cunha and Dobzhansky 1954 paper and in table 5 in the present article. For explanation of these ratings see Dansereau, 1952 and da Cunha and Dobzhansky, 1954. Knowing the degrees of the polymorphism (i.e., the mean numbers of inversions per individual) and the environmental ratings for a series of localities, we have decided to make collections in several new localities, which we have selected because we could make some predictions concerning the extent of the polymorphism to be expected there (table 5). Such predictions are based on the evaluations of the climatic and biotic conditions, and of the fidelity (see Dansereau, 1952 for explanation of this concept), in the geographic locations selected. Obviously, we could not predict before making the collections whether species of Drosophila competing with *D. willistoni* will or will

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not be frequent in a given locality. The predictions take, therefore, something like the following form: "If *D. willistoni* is the dominant species of its group in the locality X, then, on the basis of the other environmental ratings, we expect about Y inversions per individual there."

Our first prediction concerned the population of Rio Verde, Mato Grosso, No. 49 in figure 2 and in tables 2–5. This locality is about midway between Goias (localities Nos. 53 and 55) with environmental ratings of 12 and 11, and with more than 9 inversions per individual, and Porto Velho and Acre (localities Nos. 45–47) with environmental ratings of 9, and 4.7–6.4 inversions per individual. The environment of Rio Verde is more similar to that of central Goias than to that of Acre. We have, therefore, concluded that, if *D. willistoni* is the dominant species in the region of Rio Verde, its populations there should have about as many inversions as in Goias, and more than in Acre. The collection has verified the prediction, the number of inversions observed in Rio Verde being 9.15 ± 0.25, the total environmental rating, including the rating of competitors, being 12.

To the south of the highly polymorphic Goias populations, in the States of São Paulo and Rio de Janeiro, there occur populations with 2.9–4.1 heterozygous inversions per individual and en-

TABLE 5. *Environmental ratings and mean numbers of heterozygous inversions per female in certain populations from continental South America*
Newly studied populations are marked by asterisks

	Climate	Biotic	Competitors	Fidelity	Total rating	Inversions
*24. Villavicencio	TU-4	4	3	1	12	8.65 ± 0.21
27. Içana	TiŪ-2	4	2	1	9	6.74 ± 0.56
28. Foz de Caiari	TiŪ-2	4	1	1	8	5.29 ± 0.51
29. Rio Branco	TU-4	3½	3	0	10½	4.77 ± 0.43
30. Mucajai	TŪ-3	3	1	1	8	2.67 ± 0.46
31. Rio Negro	TiŪ-2	3	2	1	8	5.72 ± 0.34
32. Fordlandia	TŪ-4	3	2	1	10	5.88 ± 0.20
33. Amapa	TŪ-3	4	3	1	11	4.75 ± 0.21
34. Marajo	TŪ-3	1	1½	0	5½	1.67 ± 0.19
35. Belem	TŪ-3	3	2	1	9	3.16 ± 0.16
36. Castanhal	TŪ-3	3	2	1	9	4.42 ± 0.34
*37. São Luiz	TU-3½	3	3	1	10½	5.58 ± 0.25
*43. Tarapoto	TŪ-3	4	3	1	11	6.18 ± 0.28
*44. Tingo Maria	TŪ-3	4	3	1	11	6.52 ± 0.26
45. Rio Moa	TŪ-3	3	2	1	9	4.68 ± 0.26
46. Palmares	TŪ-3	3	2	1	9	5.49 ± 0.31
47. Porto Velho	TŪ-3	3	2	1	9	6.39 ± 0.66
*48. Santa Cruz	tu-2	3	3	1	9	4.72 ± 0.32
*49. Mato Grosso	TU-4	4	4	0	12	9.15 ± 0.25
50. Imperatriz	TU-4	4	4	0	12	5.47 ± 0.37
51. Carolina	TU-4	4	3	0	11	8.65 ± 0.58
52. Floriano	TU-3½	2	3	0	8½	5.14 ± 0.33
53. Palma	TU-4	4	4	0	12	9.11 ± 0.44
54. Barreiras	Tu-3	3	3	0	9	7.36 ± 0.40
55. Monjolinho	TU-4	4	3	0	11	9.34 ± 0.26
62. Boa Esperanza	tu-3	2	4	0	9	4.34 ± 0.25
63. Rio de Janeiro	TiŪ-2	2	2	1	7	2.91 ± 0.22
*64. Angra dos Reis	TiŪ-2	2	2	1	7	3.52 ± 0.10
*65. Araguari	TU-3½	3	4	0	10½	7.12 ± 0.20
66. Pirassununga	tu-3	2	4	0	9	4.13 ± 0.10
67. Mogi	tU-3	2	2	0	7	3.97 ± 0.13
68. Vila Atlantica	TiŪ-2	2	2	1	7	3.06 ± 0.12

vironmental ratings of 7–9 (localities Nos. 63, 64, 66–68 in fig. 2 and table 5). We have chosen to collect in the territory known as the "Triangulo Mineiro" (locality Araguari, No. 65, see fig. 2 and tables 2–5). This locality is about midway between the Goias and the São Paulo-Rio de Janeiro localities, but its environmental rating is 10½, i.e., somewhat closer to those of the Goias localities. We have predicted that the polymorphism will be intermediate or closer to that in Goias. The figure observed, 7.12 ± 0.20 is in accord with the prediction. Dr. M. Wasserman's collection near Santa Cruz, Bolivia, was most welcome since we wished to test the expectation that the polymorphism there will be lower than in Goias, on Rio Verde, or at Araguari (the sample from Bolivia available to da Cunha, Burla, and Dobzhansky, 1950, was too small to be relied upon). The prediction has been verified, with the environmental score of 9 and the mean of 4.72 ± 0.32 inversions per individual.

Distributional Margins

Previous work has shown that in *D. willistoni* the extent of the polymorphism decreases towards the margins of the geographic distribution region (da Cunha, Burla, and Dobzansky, 1950; Townsend, 1952; da Cunha and Dobzhansky, 1954; Dobzhansky, 1956). We reasoned, however, that the distribution margins must be of two kinds. In the absence of physical barriers to its spread, a living species will gradually diffuse from territories in which the environment is highly favorable for its life, to submarginal and marginal territories in which it will be able to hold out in only a limited range of ecological niches. When this happens, the polymorphism, and particularly the chromosomal polymorphism due to inversions, may be expected to dwindle from the central to the marginal territories. On the other hand, a species may reach an overpowering obstacle, such as (for a land animal) a sea coast or a mountain range. Environments highly propitious to its life, which it has thoroughly mastered in the process of adaptive evolution, may stop abruptly at the obstacle. With distribution margins of this sort, one may expect populations as polymorphic as those in the center of the range to reach the margins.

Drosophila willistoni in South America flourishes most in tropical rainforests, and especially where these interdigitate with rich savanna vegetations. Both south and north of the Equator, such conditions extend in many places up to the eastern slope of the mighty chain of the Andes, and the rainforests of the lower parts of the Andean slope are, in fact, remarkably luxuriant and rich in species of trees, epiphytes, and lianas. *D. willistoni* does not, however, live in the mountains themselves or on the western slope. Its upper limits are not well known, but at least in Colombia we have not found this species in the cloud forest zone or above. The Andean chain is an obstacle for its spread. In 1955, we collected at Tarapoto and at Tingo Maria, in Peru, and in 1958 near Villavicencio, Colombia, expecting to find there highly polymorphic populations, despite the geographically marginal nature of these localities (Nos. 24, 43, and 44 in fig. 2 and tables 2–5). The environmental ratings for these localities come up to 12 for Villavicencio and 11 for Tarapoto and Tingo Maria. The mean numbers of the inversions proved to be 8.65 ± 0.21, 6.18 ± 0.28, and 6.52 ± 0.26 respectively. The polymorphism in these marginal localities is, thus, greater rather than smaller than in the more centrally lying collecting places in the basin of the Amazon (Nos. 27–31, 45 and 46, fig. 2).

In Colombia the Andes become split into three chains, known respectively as Cordillera Oriental, Central, and Occidental. Tropical forests and savannas penetrate between the Cordilleras and around their low northern ends, joining the forests of Panama and of the Pacific Coast, which has here a very heavy rain-

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fall. The forests extend along the coast into Ecuador, but the rainfall dwindles rapidly, giving place, south of the Gulf of Guayaquil, to the coastal desert of Peru. *D. willistoni* is distributed continuously from the Cordillera Oriental eastward, but it also sends finger-like projections up the valleys between the Cordilleras and along the coast. Environmental ratings of the same sort which we have used for the continuous distribution would hardly be meaningful for the distributional pockets, but it can be said that at least along the coasts the environment is favorable for *D. willistoni*.

Figures 1 and 2, and tables 1–4, show remarkable differences between the populations of this region. We have already considered above the populations of the eastern slope of the Andes (Nos. 24, 43, 44) which show very high polymorphism. In the north of Columbia and in Panama, where the mountains are relatively low, or can be by-passed by *D. willistoni*, high polymorphism likewise prevails (Nos. 16–19, 6.18 to 7.2 inversions per individual). But near Palmira, in the upper part of the Cauca Valley (No. 23) a population has been found having only 2.23 ± 0.18 inversions per individual, and apparently lacking several kinds of inversions very common east of the Andes. Professor Marshall Wheeler informs us that he found no *D. willistoni* only a short distance up the Cauca Valley, near Popayan.

An even sharper drop in the chromosomal polymorphism occurs as one proceeds southward along the Pacific Coast of Colombia and Ecuador. In the luxuriant rainforests near Buenaventura (No. 22) we found only 2.11 ± 0.38 inversions per individual, while in the rainforests of coastal Ecuador the polymorphism dwindles to 1.17 ± 0.38 and even to 0.44 ± 0.13 inversions per individual (Nos. 25 and 26). Here, then, we observe populations nearly as monomorphic as those of oceanic islands, yet living in close proximity to some of the most highly polymorphic ones (e.g., Villavicencio, No. 24) but separated from the latter by the apparently insuperable barriers of high mountains in which the species does not live. And yet the environment which these nearly monomorphic populations inhabit is so luxuriant that a high polymorphism would be expected if this region were a part of a continuous continental distribution. Unfortunately, no collections have been made on the coast of northern Peru (e.g., in the Tumbes region); it is not probable that *D. willistoni* would be encountered there.

The samples which we had from the not previously studied islands of the Lesser Antilles, namely, St. Thomas and St. Vincent (Nos. 7 and 10, tables 1, 3, and 4), were very small. They agree, however, with the older results which showed very little polymorphism in the populations of other Lesser Antilles. Similarly, the sample from Tikal, in northern Guatemala, had as few or fewer inversions as in the populations of Honduras and Salvador (localities Nos. 12–14, fig. 1 and tables 1, 3, and 4). Unfortunately, we do not have adequate samples from the hot country of central Mexico, where the species reaches its continental northern limit, and where one would expect perhaps even fewer inversions.

Northeastern Brazil

In 1954, da Cunha and Dobzhansky described population samples from 6 localities in northeastern Brazil (Nos. 52, 56–58, 60, 61, fig. 2). Among these, Nos. 56 and 57 are in the arid zone where *D. willistoni* is not a successful species, being heavily outnumbered by *D. nebulosa*, while other sibling species of *willistoni* group are altogether absent. With environmental rating of 6 and 7, a low degree of polymorphism is expected and is observed (about 1 inversion on the average per female). However, the localities Nos. 58, 60, and 61 lie in the coastal tropical forests in which *D. willistoni* prospers greatly. With environ-

mental scores of 9, highly polymorphic populations are expected, yet the polymorphism observed is both quantitatively and qualitatively similar to what is found in the arid desert zone. Only in the locality No. 52, which represents an ecotone between the Northeast and the savanna forest region of central Brazil, the polymorphism goes up to 5.14 ± 0.33 inversions per female; with an environmental score of 8½, this is about right for the South American continental populations considered as a whole.

We have endeavored to collect material in hitherto unstudied parts of northeastern Brazil, in order to discover the extent of the anomaly previously observed. Collections were made in the localities Nos. 37–42, as shown in figure 2 and tables 2–4. Of these, No. 37 lies in the equatorial rainforests of coastal Maranhão, but only some 400 km. from the ecotone with the arid zone of Ceara, and about equally far from previously studied ecotonal locality No. 52. The environmental rating of No. 37 comes up to, at least, 9 (more likely 9½), and the two samples from there (table 3) had 5.58 ± 0.25 and 6.05 ± 0.36 inversions per individual, which is about the expected magnitude. Qualitatively, the inversions found in No. 37 resemble No. 52 and the adjacent equatorial zone of Para (Nos. 35 and 36, fig. 2).

Localities Nos. 38–42 are in the arid zone of the Brazilian Northeast, although Nos. 38 and 39 are in the relatively humid but isolated montane forests of the "Serras" rising above the deserty plain. Nos. 40–42 are on the coast, where the aridity of the interior is somewhat mitigated, but in conditions much resembling the old locality No. 57. The environmental ratings of these localities are estimated at 6½ (No. 38) or 7 (Nos. 39–42), and the mean numbers of heterozygous inversions are between 1.17 ± 0.12 and 1.79 ± 0.23 (table 3), which is again in agreement with the expectation. The findings contradictory to the hypothesis in the localities Nos. 58, 60, and 61 thus remain unique and unexplained. The coastal rainforests in which these localities are situated come up from the south as a gradually narrowing strip which disappears south of the locality No. 57. Nevertheless, the situation here is not comparable with that on the coasts of Colombia and Ecuador, where a distributional pocket is formed isolated from the east by the high Andes. The coastal hills in Brazil do not constitute a barrier for *D. willistoni;* on the contrary, this species is quite common in these low mountains (locality No. 60, fig. 2).

Discussion

Our working hypothesis has been that one of the methods whereby a living population may achieve a mastery of its environment is by means of adaptive polymorphism. This hypothesis is a corollary to the simple consideration that no one genotype is likely to be superior to all others in all ecological niches which are potentially accessible to a Mendelian population in a given geographic region. Successful settlement of these niches and exploitation of their adaptive opportunities may, therefore, be facilitated by genetic diversification. The evidence derived from studies on *Drosophila willistoni* is on the whole consistent with the hypothesis. But even in this species there is an exception—the populations of the coastal rainforcests in Bahia and Espirito Santo, Brazil (localities Nos. 58, 60, and 61, fig. 2), which are apparently quite successful in their bountiful and varied environments, and yet show little polymorphism. Consistent results have also been reported for other species of Drosophila by da Cunha, Brncic and Salzano (1953), Salzano (1955), and Carson (1955, 1956). Goldschmidt and Stumm-Zollinger (1959), working on *D. subobscura,* have been impressed by exceptions more than by consistencies.

Concerning the exceptions, some of which we have discovered in our material as well, this much needs to be

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said. The hypothesis certainly does not claim that polymorphism is the sole method of adaptation to complex environments. We have no more right to expect that all flourishing species and populations will be chromosomally polymorphic than we do to expect all animals to be protectively colored, or all to exhibit mimetic resemblances. The absence of protective coloration in some species and of mimetic resemblance in others does not, however, invalidate the hypotheses that such colorations and resemblances are important adaptive devices. Although the intrapopulational adaptive polymorphism takes in many species of Drosophila the form of balanced chromosomal polymorphism, in other species, particularly of the subgenus Drosophila, it does not. Thus, the very successful and widely distributed oriental species *D. virilis* is chromosomally monomorphic. Surely, this does not mean that it is also genetically monomorphic.

In a brilliant series of investigations, Carson (1955a, b, 1956, 1958) has restated the hypothesis in terms which are superior to our original ones, particularly when applied to the chromosomal polymorphism in Drosophila in its relations to the environment. His argument starts with the consideration that in populations in which most individuals are heterozygous for several inversions, the recombination of genes by crossing over will occur less freely than in chromosomally monomorphic populations. Highly polymorphic populations may thus be regarded as having reached a relatively stable state of high adaptedness, but at the same time of specialization, to the varied ecological niches which they have thoroughly mastered in a long-occupied geographic territory. Geographically marginal but chromosomally monomorphic populations may have mastered fewer habitats, but in compensation they enjoy the advantages of freer recombination, and consequently of higher adaptive plasticity. Therefore, "regional differentiation of chromosomal characteristics, with relative structural homozygosity at the margin, thus provides a solution to the ancient evolutionary dilemma with which the organism is continually faced. This is the maintenance of a high adaptive level and at the same time the preservation of a balance between too much recombination, which may destroy adaptive gene complexes, and too little, which restricts the ability of the organism to change genetically in response to drastically changed conditions" (Carson, 1955b).

The populations studied by Carson have been those of *D. robusta*. For this species Carson has developed a method of characterization of geographic populations not in terms of the average frequencies of inversion heterozygotes which we have used, but in terms of proportions of the total chromosome length in which crossing over is free to occur and in which it is effectively suppressed. We have attempted to estimate such recombination indices also for the populations of *D. willistoni*. The values so obtained stood in about the same proportions to each other as did the frequencies of the inversion heterozygotes shown in figures 1 and 2. This is as it should be; the two measures could be discrepant only if the inversions found in some populations were on the average much longer than those found in other populations. In *D. willistoni*, most inversions found in nature are rather short; in some other species, such as *D. pseudoobscura* and *D. persimilis*, they are rather long. Although our working hypothesis and that of Carson emphasize somewhat different aspects of the situation, they are complementary rather than alternative.

The same cannot be said of the opinion set forth with, perhaps, needless aggressiveness, that the adaptedness of Mendelian populations is not promoted by polymorphism. This has been answered very effectively by Li (1955b). We may only recall that Ludwig has discussed in a little known paper (1950, see also Ludwig, 1954) the phenomenon which

he has called "annidation." A mutant which can exploit an adaptive niche not accessible to the ancestral type will be retained in the population even if it is inferior to its ancestor in the niche, or niches, accessible to both. A population containing the mutant as well as the ancestral type will then have available to it more opportunities for life ("Existenzplätze") than the ancestral populations had. "Annidation" is regarded by Ludwig as one of the main adaptive processes in evolution. Levene (1953), Li (1955a), and Dempster (1955) have examined mathematically various conditions which will lead to stable equilibria of polymorphs, with and without heterozygotes being heterotic, in a Mendelian population which has mastered a variety of ecological niches. The quality as well as the quantity of polymorphism in a Mendelian population is thus regulated by natural selection. In what sense it may be said that natural selection promotes the adaptedness, or Darwinian fitness, or selective value, of Mendelian populations is quite another matter, for discussions of which see Thoday (1953, 1958), Dobzhansky (1955), and others.

Summary

Data are given on the quality and quantity of the chromosomal polymorphism in certain populations of Central and South America which have not been previously studied. The over-all picture that emerges is summarized in figures 1 and 2.

Some of the populations are highly polymorphic and other predominantly monomorphic. Populations of the central part of the geographic distribution area of the species tend to be more polymorphic than those of the peripheral parts and distributional pockets; populations which occupy rich and diversified environments are more polymorphic than those of submarginal and marginal environments; and populations which face the competition of closely related species are more monomorphic than those enjoying a near-monopolistic status. These regularities suggest that the chromosomal polymorphism is, in *Drosophila willistoni*, a method whereby populations may achieve a mastery of their environments. Using this working hypothesis, we have attempted to predict the extent of the polymorphism expected in populations of certain hitherto not studied localities. Such predictions have been verified by observation. Nevertheless, the populations of a part of coastal Brazil constitute an exception, being less polymorphic than they might have been expected to be.

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Literature Cited

Carson, H. L. 1955a. The genetic characteristics of marginal populations of Drosophila. Cold Spring Harbor Symp. Quant. Biol., **20**: 276–287.

——. 1955b. Variation in genetic recombination in natural populations. J. Comp. Cell. Physiol., **45**, Supp. 2: 221–236.

——. 1956. Marginal homozygosity for gene arrangement in *Drosophila robusta*. Science, **123**: 630–631.

——. 1959. Response to selection under different conditions of recombination in Drosophila. Cold Spring Harbor Symp. Quant. Biol., **23**: 291–306.

DA CUNHA, A. B., D. BRNCIC, AND F. M. SALZANO. 1953. A comparative study of chromosomal polymorphism in certain South American species of Drosophila. Heredity, **7**: 193–202.

——, H. BURLA, AND TH. DOBZHANSKY. 1950. Adaptive chromosomal polymorphism in *Drosophila willistoni*. Evolution, **4**: 212–235.

——, AND TH. DOBZHANSKY. 1954. A further study of chromosomal polymorphism, in *Drosophila willistoni* in its relation to the environment. Evolution, **8**: 119–134.

DANSEREAU, P. 1952. The varieties of evolutionary opportunity. Rev. Canad. Biol., **11**: 305–388.

DEMPSTER, E. R. 1955. Maintenance of genetic heterogeneity. Cold Spring Harbor Symp. Quant. Biol., **20**: 25–32.

DOBZHANSKY, TH. 1950. The chromosomes of *Drosophila willistoni*. J. Heredity, **41**: 156–158.

——. 1957. Genetics of natural populations, XXVL. Evolution, **11**: 280–293.

GOLDSCHMIDT, E., AND E. STUMM-ZOLLINGER. 1959. Geographical differentiation of inversion systems in *Drosophila subobscura*. Evolution, **13**: 89–98.

LEWONTIN, R. C. 1955. The effects of population density and composition on viability in *Drosophila melanogaster*. Evolution, **9**: 27–41.

LEVENE, H. 1953. Genetic equilibrium when more than one ecological niche is available. Amer. Natur., **87**: 311.

LI, C. C. 1955a. Population genetics. 2nd ed. Chicago.

——. 1955b. The stability of an equilibrium and the average fitness of a population. Amer. Natur., **89**: 281–295.

LUDWIG, W. 1950. Zur Theorie der Konkurrenz. Die Annidation (Einnischung) als funfter Evolutionsfaktor. Neue Erg. Probl. Zool., Klattfests., 516–530.

——. 1954. Die Selektionstheorie. In Heberer's "Die Evolution der Organismen," 2 Auf., 662–712.

SALZANO, F. M. 1955. Chromosomal polymorphism in two species of the Guarani group of Drosophila. Chromosoma, **7**: 39–50.

TOWNSEND, J. I. 1952. Genetics of marginal populations of *Drosophila willistoni*. Evolution, **6**: 428–442.

——. 1958. Chromosomal polymorphism in Caribbean Island populations of *Drosophila willistoni*. Proc. Nat. Acad. Sci., **44**: 38–42.

MESOZOIC MAMMALS AND THE POLYPHYLETIC ORIGIN OF MAMMALS

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This is, in one way, a poor time to review the Mesozoic mammals and the origin of mammals. Discoveries and studies announced but not yet published fully, if at all, are extraordinarily rich and important. They include: late Cretaceous mammals from Wyoming (McKenna and associates at the University of California); Triassic mammals or very advanced mammal-like reptiles from England (Kermack and Mussett; preliminary notes have been published), China (Olson and Patterson) and South Africa (Crompton; the first of a series of studies has been published); and tritylodonts from Arizona (Lewis and Colbert). When available these prospective works will throw a flood of new light on the whole subject and will certainly greatly modify any conclusions that can now be reached. On the other hand, this pleasing prospect (complete fulfillment of which may be long delayed) makes it all the more interesting to survey what we do know now and what it may mean, to look at the base for the next advances. There are also several recent publications of major importance that merit review and that suggest reconsideration of various points, especially: Patterson (1956), Peyer (1956), Kühne (1958), Crompton (1958), and Kermack and Mussett (1958 a, b).

This review, centered on the affinities of the known forms, will consider those forms in sequence from later to earlier:

Late Cretaceous. Although this situation is due to change rapidly, at present the published data on late Cretaceous mammals have not been really significantly increased in the last thirty years. The known specimens are from Mongolia (Simpson, 1928d, and references there) and North America (especially Simpson, 1929b; later studies by me and others so far add little of real importance). All fall into two very clear-cut groups, multituberculates and Theria with tribosphenic molars. The latter include unquestionable marsupials (e.g., *Eodelphis*) and placentals (e.g., *Zalambdalestes*). Some of the marsupials are closely similar to living opossums in the dentition and lower jaw. In spite of considerable diversity they represent or include very primitive forms from which any or all later marsupials could be structurally derived. The placentals are generally classed as Insectivora. They are, however, such not in the same sense as the later, specialized insectivores, but in the sense that they lack known clear-cut ordinal characters within the Theria and that "Insectivora" has been used as a convenient catch-all for such virtually nonordinal groups. Their degree of diversity and any special relationships to known later groups are yet to be established.

Early Cretaceous. Endotherium Shikama, 1947, was described as a placental mammal from the Jurassic of South Manchuria. Kühne (1958) accepted Shikama's views as to age and affinities. He concluded that placentals arose in the Jurassic from pantotheres, and that marsupials arose in the early Cretaceous from placentals. Patterson (1956),[1] however, pointed out that *Endotherium* is probably

[1] Kühne had not received this publication by Patterson when he wrote the manuscript of Kühne (1958). He added a note on Patterson (1956) while his paper was in press, but did not mention Patterson's views on *Endotherium*.

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of early Cretaceous age and that while the lower molars are more advanced than in Jurassic Pantotheria the known parts are not diagnostic as between Marsupialia and Placentalia. It is, indeed, uncertain whether marsupials and placentals were then really distinct and Patterson believes that they were not.

Patterson (1956 and earlier notes there cited) described a small but varied mammalian fauna from the early Cretaceous of Texas. The fauna is from well up in the lower Cretaceous rocks of usual American usage, and would be middle Cretaceous for those who accept a tripartite division of the period. The multituberculates, not yet fully described, are said to be nearer those of the late Jurassic (Plagiaulacidae) than those of the late Cretaceous. (A plagiaulacid is also known from the earlier Wealden Cretaceous of England; Simpson, 1928a.) A symmetrodont and a triconodont are survivors of orders otherwise known only from the Jurassic. The therians are represented by specimens so fragmentary that they cannot be exactly classified. They are more advanced than Jurassic Pantotheria and are, in Patterson's expression, of "Metatherian-Eutherian grade." Patterson inclines to the view that they are ancestral to both Metatheria (Marsupialia) and Eutheria (Placentalia) and might represent an order otherwise unknown (except perhaps for *Endotherium*) referable to an infraclass Pantotheria but not the order Pantotheria.

Late Jurassic. No really important new observations on late Jurassic mammals have been made since my old monographs (Simpson, 1928a, 1929b), but there has been considerable further discussion of their affinities and cusp homologies, especially Butler (1939), Bohlin (1945), Patterson (1956), Kühne (1958), and Kermack and Mussett (1958b). I (1928a) recognized four orders, Multituberculata, Triconodonta, Symmetrodonta, and Pantotheria, which I considered essentially unrelated, perhaps independently derived from reptiles, except for a possible rather remote special relationship between Symmetrodonta and Pantotheria. Butler (1939) did not treat the Multituberculata and arranged the others, along with later mammals, in three grades: first, Triconodonta and Symmetrodonta, believed to be especially related; second, Pantotheria, divided into quite distinct groups Docodontoidea and Dryolestoidea, and the Cenozoic zalambdodont insectivores (except *Solenodon*)[2]; third, the post-Jurassic marsupials and placentals (except zalambdodonts). Bohlin (1945) recognized the same four independent orders as in Simpson (1928a), but believed that the multituberculates and symmetrodonts were derived from unknown ancestral triconodonts, probably still reptilian by definition, while the pantotheres were of quite separate origin.

Patterson (1956) emphasized Butler's distinction of Docodontoidea and Dryolestoidea by removing the former altogether from the Pantotheria and making them a separate order Docodonta, of independent origin from Therapsida. He emphasized and extended my very tentative collocation of Symmetrodonta and Pantotheria (now minus the docodonts), with the opinion that pantotheres were derived from symmetrodonts. Multituberculata, Triconodonta, Docodonta, and Symmetrodonta plus Pantotheria were believed to represent different crossings of the grade-line Reptilia-Mammalia. In a work essentially contemporaneous with and independent of Patterson's, Kühne (1958) argued that pantotheres (including docodonts) were derived from Liassic triconodonts, while symmetrodonts and multituberculates

[2] The zalambdodont insectivores are clearly placentals (Eutheria) and can hardly have had an origin in the Jurassic separate from that of the other Eutheria or the common Metatheria-Eutheria stock. Butler (1956) later agreed with this implicitly by placing the zalambdodonts in the Soricoidea, a specialized Cenozoic superfamily of Insectivora (*sensu stricto*), along with the shrews.

were each independently derived from reptiles. Kermack and Mussett (1958b) accept and emphasize separation of docodonts from (true) pantotheres. They somewhat equivocally unite docodonts with some supposedly pre-monotreme Rhaetic-Liassic forms discussed below.

Views as to affinities among the late Jurassic orders are thus contradictory. I would now accept Patterson's [3] ordinal separation of the Docodonta. As to affinities between any two or more of the five orders (even that between Symmetrodonta and Pantotheria), I feel that no one has really made out a good case. They probably represent at least three (but which three?) and possibly five different attainments of the mammalian grade by different lineages of former reptiles. The solution will come when Rhaetic-Liassic groups are better known.

Middle Jurassic. The only known middle Jurassic mammals are a few from Stonesfield, England. No new discoveries have been made since well before I reviewed the fauna (Simpson, 1928a). The small fauna includes triconodonts and a pantothere (*sensu stricto*, "dryolestoid" not docodont). Symmetrodonts were certainly, docodonts almost certainly, and multituberculates probably in existence at that time so their absence is due either to facies or to the inadequacy of the sample. Associated with the undoubted mammals is a tritylodont (*Stereognathus*), either the latest known therapsid reptile or another quite distinct lineage into the grade Mammalia (see below).

What is a Mammal? This question becomes essential already in the middle Jurassic, and increasingly in earlier beds. Through the Triassic the mammallike reptiles advanced so steadily toward mammalian status, and early mammals, although less well known, were still so reptilelike in some features that it has become obvious that achievement of the mammalian grade was a long and gradual process. The definition of that grade must, therefore, be to some extent arbitrary. The combinations of characters distinguishing most or all recent mammals from recent reptiles cannot be used for such definition. It is, for instance, obvious even from living forms (monotremes) that in some lineages, at least, the origin of mammary glands preceded viviparity so that one or the other, but not both, could be used in arbitrary definition of the reptile-mammal line. In fact, as a practical matter neither one can be used, because those characters are very rarely observable in fossils and the actual transitions for which the definition is needed are all extinct. These well known difficulties long since led to agreement to base the arbitrary definition on the articulation of mandible and skull and associated osteological features.

Until recently the following diagnoses were generally accepted:

Reptile

1. Articular-quadrate suspensorium
2. No dentary-squamosal joint
3. One bone (stapes) in middle ear
4. Mandible compound

Mammal

1. Articular-quadrate not a suspensorium
2. Dentary-squamosal joint
3. Three ossicles in middle ear
4. Mandible of one bone (dentary) only

These are functionally and anatomically related characters and in recent animals they are always associated as in the foregoing diagnoses. That was long true also of fossils in which these elements were known. Nevertheless these are four different characters, not one, and it should not have been assumed that they necessarily changed simultaneously from the reptilian to the mammalian condition. In fact there is now increasing evidence that they did not (Crompton, 1958; Kermack and Mussett, 1958 a, b; Kühne, 1958). There is direct evidence of animals (to be specified below) that had both articular-quadrate and dentary-

[3] First nomenclatural proposal of an order Docodonta was actually by Kretzoi, but on inadequate grounds and without personal study of any of the forms involved.

squamosal articulation of mandible and skull and that were, therefore, reptiles by criterion (1) and mammals by criterion (2). The change in criterion (3), not directly known in materials so far published, necessarily was later than (1) and either concomitant with or later than (2). Change in (4) was necessarily later than (1) and concomitant with or later than (2). It would probably, but not with absolute *a priori* necessity, also be concomitant with or later than (3). Again there are no really decisive published observations, but there is some indirect evidence that in some groups change (4) was considerably later than (3). Moreover the evidence suggests that it is highly improbable that the four changes occurred at the same rates or times or even in the same sequence in all the lineages that were becoming mammalian in grade.

So now what is a mammal? Kühne (1958) concludes that there is no dividing line between therapsids and mammals; the "Typus Mammalier" is simply an artifact. He does not explicitly go so far, but the logical inference would seem to be that we must either classify therapsids as mammals (which was in fact suggested long since) or perhaps still better classify "mammals" as reptiles. Crompton (1958) holds that an animal reptilian in criterion (1), hence also necessarily in (3) and (4), should be classified as a reptile even though it is a mammal by criterion (2). Kermack and Mussett (1958 a, b), on the other hand, give priority to (2) "for practical convenience," and would classify as Mammalia all "synapsids" with a dentary-squamosal joint even though they were reptilian as regards (1), (3), and (4). It would, in principle, be equally logical to select either (3) or (4) as the arbitrarily diagnostic character. I agree, however, with Kermack and Mussett that (2) is at present the most practical criterion, simply because it can most often be unequivocally established for the fragmentary materials actually in hand.

There are numerous other dental and osteological characters that are universally present in animals generally considered to be primitive mammals, e.g., cusp differentiation on cheek teeth, separation of roots on cheek teeth, absence of postorbital bar, presence of secondary palate, and many others. But all these characters also occur in some animals now universally classed as reptiles and there would be no advantage in using them as criteria for definition of the classes. They are of some use in estimating probable affinities when the jaw-ear characters are unknown.

By any of these criteria the Mammalia are a grade, not a clade, in Huxley's (1958) terminology. That is, none of the definitions now current makes the Mammalia strictly monophyletic, a point discussed below.

Late Triassic-Liassic. The most mammallike forms of the late Triassic (especially Rhaetic) and the Liassic are similar in various finds scattered in space and time and no significant phyletic or structural sequences have been established among them, so they may be treated together. They are described, with references to earlier publications, especially in the following: Simpson (1928a and b), Broili and Schroeder (1936), Young (1947), Parrington (1947), Kühne (1950), Peyer (1956), Kühne (1956), Kühne (1958), Crompton (1958), and Kermack and Mussett (1958 a, b). As regards the cheek teeth, at least, most of these finds fall into four main morphological groups:

(a). Cheek teeth more or less triconodontlike or predocodontlike. Morganucodontidae and perhaps other families.

(b). Cheek teeth symmetrodontlike. Referred to the Symmetrodonta.

(c). Cheek teeth with central basin and cuspidate rims. Haramyidae.

(d). Cheek teeth with two (below) or three (above) rows of more or less crescentic cusps. Tritylodontidae.

To these may be added (e) the

Ictidosauria, cheek teeth unknown in some, in others not yet adequately described. There are also a few other poorly known groups that are of doubtful position somewhere near the reptile-mammal transition, e.g., the so-called Protodonta (Simpson, 1926) which may belong in (a) as Kühne thinks, or (e) as classified by Romer (1945), or may simply be advanced therapsid reptiles.

In group (a) the most important finds are the similar and probably closely related animals called *Eozostrodon* by Parrington (1947 and earlier), called *Morganucodon* by Kühne (1958 and earlier), described by Peyer (1956) without a name, and announced but not yet adequately described by Kermack and Mussett (1958a, b). The first three publications cited are based on isolated teeth; the last two announce but only in small part describe the discovery of associated dentitions, jaws, and other elements. Parrington and Peyer considered those animals Triconodonta. Kühne (1949) at first called his *Morganucodon* a triconodont and then (1950) a pre-pantothere, citing P. M. Butler's otherwise unpublished opinion that it was a "predocodontid." (Both Kühne and Butler then considered the docodonts as pantotheres.) Patterson (1956) sharply separated the docodonts from (other or true) pantotheres and considered *Morganucodon* a docodont, hence in his opinion nothing to do with pantotheres and perhaps connected with triconodonts only remotely, in a premammalian stage. Kühne (1958) returned to the view that morganucodonts are triconodonts, but quite distinct from later Triconodonta. Except in making morganucodonts also ancestral to some triconodonts, this is not radically different from his opinion of 1950, because Kühne still considered morganucodonts as ancestral to the Pantotheria (including docodonts). In 1958 he described a number of upper teeth which are, indeed, much less docodontlike and distinctly more triconodontlike than the lower teeth known (by publication) to Patterson.

Kermack and Mussett (1958b) have partially described and illustrated the petrosal and the posterior part of the jaw in their "morganucodonts" (or eozostrodonts, as *Eozostrodon* antedates and is perhaps synonymous with *Morganucodon*). They have discussed the dentition in general terms but as yet without descriptive detail. The jaw structure is similar to that of the late Jurassic *Docodon* and quite unlike any other later forms. On that basis Kermack and Mussett tentatively place these Rhaeto-Liassic forms in the Docodonta, although these early forms are not considered likely ancestors of the later docodonts. The teeth, on the other hand, (particularly the upper teeth) are quite unlike those of *Docodon* and more, although not exactly, like those of triconodonts. Relationships among these three groups, "morganucodonts," docodonts (*sensu stricto*), and triconodonts, are not resolved.

It is not quite certain but is considerably more likely than not that "morganucodonts" and docodonts retained a compound lower jaw with a functional articular bone (not yet properly a malleus; Kühne, 1958; Kermack and Mussett, 1958a, b). They had, nevertheless, a strong dentary-squamosal articulation. They were therefore mammals by criterion (2) of the preceding list, probably reptiles by the other criteria. Kermack and Mussett propose a new subclass "Eotheria" for animals in this condition. Since, however, all mammals probably went through this stage and several of their lineages did so independently (as Kermack and Mussett recognize), the "subclass" probably has no phylogenetic validity and its usefulness as a grade is, in my opinion, dubious.

In group (b) Kühne (1950 and 1958) has described one tooth and mentioned two others. Kermack and Mussett (1958a) have further and evidently much better material. There seems to be little

doubt that these are indeed ancestral symmetrodonts. Kermack and Mussett state that the Rhaeto-Liassic forms had a double jaw articulation, like their Rhaeto-Liassic "docodonts" or "morganucodonts." The late Jurassic symmetrodonts clearly had no articular-quadrate suspensorium and so presumably were also mammals by criterion (3) as well as (1) and (2). (It is possible, but quite improbable, that in some of these groups the articular and quadrate lost function as suspensorium and still did not become middle ear ossicles.) The late Jurassic forms still had the internal mandibular groove (Simpson, 1928c), which in the opinion of Kühne (1956) and of Kermack and Mussett (1958) indicates retention of a separate splenial bone. This is probable but there are some anomalies and it must remain somewhat uncertain unless the bone itself is discovered. If it was present, the late Jurassic symmetrodonts were mammals by criteria (1), (2), and (3) but still reptiles by criterion (4). In mid- (or late lower) Cretaceous symmetrodonts the groove is gone (Patterson, 1955) and they were mammals by all four criteria.

The present evidence almost conclusively indicates that the symmetrodonts cannot have been derived from any other known group mammalian by any definition. They cannot have given rise to any other known group except just possibly the pantotheres (as Patterson tentatively concludes). The absence of known pantotheres before the middle Jurassic is mentioned by Patterson in this and by Kühne (1958) in another connection, but the pertinent Rhaeto-Liassic forms are not so well known that negative evidence can be given much weight.

Group (c), the Haramyidae (also called Microlestidae or Microcleptidae, family names based on invalid generic names) remains mysterious. It is known only from isolated teeth (see especially Simpson, 1928a, b; Parrington, 1947; Peyer, 1956). These have two roots and good cusp differentiation, but whether the animals were mammals by any of the crucial jaw-ear criteria is unknown. This is the only known Rhaeto-Liassic group that could conceivably have given rise to the (late Jurassic to early Eocene) Multituberculata and some early students did indeed claim those affinities for them. The resemblances are, however, quite superficial and there are important differences (Simpson, 1928a; later discoveries do not significantly change the situation). The affinities of the Haramyidae and the origin of the Multituberculata are simply unknown at present.

The tritylodonts, (d), are incomparably the best-known of any of the groups reviewed here and bid fair to become among the best-known of all fossil vertebrates. Young (1947) has described fairly good skulls and jaws (*Bienotherium,* China) and Kühne (1956) assembled a nearly complete composite skeleton (*Oligokyphus,* England). Now articulated skulls and skeletons are known in abundance from Arizona (Lewis and Colbert, work announced in progress but not published). Opinion has long oscillated as to whether the tritylodonts were reptiles or mammals. From teeth and the front of a skull (*Tritylodon* itself, South Africa) but no lower jaws, ear or articular parts, or other elements, I (Simpson, 1928a) concluded that they were more mammallike in known characters than any forms then positively known to be reptiles (which was correct) and so tentatively called them mammals and accepted the possibility of distant relationship to multituberculates. The latter point was almost certainly wrong and soon had to be abandoned (e.g., Simpson, 1937).

Young (1940) at first considered his tritylodonts as mammalian, but on more detailed study concluded that the lower jaw was compound and that there was probably (with less certainty) a quadrate still involved in a suspensorium. Those animals were therefore probably reptiles

by our criteria (1), (3), and (4) and were therefore finally classed as reptiles by Young (1947). Young's material does not, however, clearly exclude the possibility that *Bienotherium* also had dentary-squamosal contact and was a mammal by criterion (2). In *Oligokyphus* Kühne (1956) established the presence of an articular-quadrate suspensorium beyond reasonable doubt. He also decided there was no dentary-squamosal contact, but that may not be quite conclusive. The absence of an incipient condyle on the dentary may not be absolutely certain with such small and fragmentary materials, and there is a glenoidlike surface in appropriate place on the squamosal—that this was a "still functionless" glenoid, as if it *anticipated* later contact with the dentary is (to me) incredible. Thus *Oligokyphus* was a reptile by criteria (1), (3), and (4), and probably but not quite surely by criterion (2).

In the latest known tritylodont (*Stereognathus,* middle Jurassic of England) neither lower jaw nor squamosal is known. Since the dentary and squamosal were at least *nearly* in contact in the Rhaeto-Liassic, it would not be surprising if they were in contact in the middle Jurassic even if not before. The point is uncertain, but it is at least possible that the tritylodonts became mammalian by criterion (2), considered diagnostic by Kermack and Mussett. If so, they did so independently, for there is no real possibility that they are related within the mammalian grade to any other known group that achieved that grade.

Broom (1929) proposed the suborder Ictidosauria, our group (e), mainly on the basis of two specimens from South Africa roughly sketched but not described in detail or named. The Ictidosauria were later used especially by Watson and Romer (history summarized with citations by Crompton, 1958) as a sort of wastebasket for a variety of animals near the reptile-mammal line and of otherwise uncertain affinities, including the tritylodonts. Crompton (1958) has now fully described the posterior parts of skull and jaw of one of Broom's specimens, now named *Diarthrognathus broomi* Crompton, which is reptilian by criteria (1), (3), and (4) but mammalian by criterion (2). (Crompton's conclusion that the animal was still a reptile is of course a matter of definition and preference.) *Diarthrognathus* and the tritylodonts were probably derived from quite different therapsid ancestors and the tritylodonts cannot now be classified as Ictidosauria. Useful comparison of *Diarthrognathus* with other Jurassic groups at or near the mammalian grade is impossible at present. Crompton has deferred discussion of possible affinities with various Triassic therapsids or therapsid-like animals sometimes called ictidosaurians, and we may best do the same.

Monotremes. The monotremes are extremely specialized in some respects, but in others, as is well known, they are at a very primitive mammalian or even a therapsid structural level. Except for scraps nearly identical with the living animals, they are unidentified as fossils. Earlier suggestions of derivation from the multituberculates are almost certainly wrong (Simpson, 1929a, 1937). The enormous gap in time between Mesozoic mammals and the Australian monotremes would perhaps permit derivation from almost any Jurassic order, but there has been no positive indication of such derivation. Now Kermack and Mussett (1958b) have shown that the petrosal of the Rhaeto-Liassic "morganucodonts" is like that of the recent monotremes and unlike that of any other known animals. They consider this as conclusive evidence of relationship and of near if not quite direct ancestry. If that is correct, I would think that the Rhaeto-Liassic forms belong in the same subclass, at least, as the monotremes, although Kermack and Mussett somewhat contradictorily place them in separate subclasses. The gap is enormous and in view of demonstrably extensive parallelism in other complex structures it is not wholly out of the question

that the resemblance in the petrosals may be due to parallelism. In any case, there is a strong consensus that the Prototheria (monotremes) arose ultimately from therapsid reptiles separately from the Theria (marsupials and placentals), whether or not the ancestry of the former is represented by the "morganucodonts."

The Polyphyletic Origin of the Mammalia. Nine separate, defined groups that did reach or may have reached mammalian status by at least criterion (2) have been discussed: Theria (including Pantotheria), Symmetrodonta, Triconodonta, Docodonta, "Morganucodontidae," Multituberculata, Tritylodontidae, Haramyidae, and Monotremata. Ictidosauria are omitted from the list because on present evidence they could have merged immediately into one of the named groups and hence are not at present a clearly separate lineage. Various other genera or small groups (e.g., the so-called Protodonta) are likewise omitted because their separate status is not now clear.

In 1928 I suggested that probably four of these groups as then known had independently crossed the structural line from reptiles to mammals: Symmetrodonta plus Theria, Triconodonta, Multituberculata, and Monotremata. As far as I know, all subsequent authors who have explicitly studied the Mesozoic mammals have agreed that they were polyphyletically derived from reptiles, although they have not agreed as to just which lineages were distinct at the transition. All agree that the Pantotheria (minus Docodonta) + Marsupialia (Metatheria) + Placentalia (Eutheria) represent a single crossing of the reptile-mammal line. All also agree that the Multituberculata represent a separate crossing.

A minimal hypothesis, which has not been fully explicit but may be implicit in Butler (1939), would associate symmetrodonts, triconodonts, and docodonts with the pantothere crossing. To keep this within the absolute minimum permitted by the data, monotremes and "morganucodonts" would be associated with the same crossing and tritylodonts and haramyids would be considered reptilian. There would be only two known crossings. Later information suggests that that number is almost certainly too small and the minimum number warranted is probably four: Symmetrodonta—Pantotheria—later Theria, Triconodonta, Docodonta (with "morganucodonts")—Monotremata, and Multituberculata. That combines views expressed by Patterson (1956) and by Kermack and Mussett (1958b). Kühne's (1958) opinion, which seems to me less probable on evidence not fully available to Kühne, would still give four: Triconodonta—Pantotheria (including Docodonta)—later Theria, Symmetrodonta, Monotremata, and Multituberculata. The maximal hypothesis that can be clearly stated on present data is that all of the groups mentioned above crossed the line separately. It is more than likely that other lineages that we do not know or have not identified in this connection also crossed the line.

The conclusion that the mammals, by structural definition, are polyphyletic is strongly supported by knowledge of the Therapsida. No attempt is made here to review the enormous literature, but Olson (1944) may be cited. The many later studies endorse and amplify his findings: through the Permian and Triassic all the Therapsids were advancing at varying rates toward mammalian structure in fundamental respects although at the same time diverging in specializations of apparently less basic character. It is not, then, surprising that different lineages actually became mammals by structural definition.

The Class Mammalia as currently recognized is thus a grade and not a clade in the terminology of Huxley (1958). That conclusion poses problems of principle for the formal taxonomy of the class. The following are not the only alternatives but are perhaps the most acceptable ones:

1. The Mammalia may be defined frankly as a grade, not a single phy-

logenetic unit, or classified typologically by structure without reference to phylogeny.

2. The Class Mammalia may be restricted to groups known (with reasonable probability) to be monophyletic *as mammals:* Pantotheria, Metatheria, and Eutheria. The other groups here discussed would then be considered either as reptiles or as *incertae sedis.*

3. The groups known to have reached mammalian grade (by whatever of the diagnoses one prefers) may be retained as a phylogenetically based Class Mammalia, on the following principles. Evolutionary or so-called phylogenetic classification does not *express* phylogeny but is *based* on phylogenetic interpretation of the observational data. It is always necessary in a formal classification (so unlike an actual phylogeny) to compromise sooner or later between horizontal and vertical separation of taxa. That each taxon sometime included only one species in its ancestry should be true, but it is a completely impractical requirement that each taxon be so delimited and defined as to include and begin with that species. In practice it is a sufficient principle for evolutionary taxonomy that each taxon arose wholly from one of lower categorical level, as Class Mammalia from Order Therapsida.

4. Some or all of the animals now called mammallike reptiles may be included in the Mammalia. This would evade the issue of polyphyly at its present level, but would probably only transfer it to an earlier level. An arbitrary structural definition would still be necessary, and such definition at the base of the Therapsida or Synapsida might be even more difficult to apply than those now used at the top of those groups. Moreover, such a radical change would make all current and earlier literature confusing and would greatly complicate teaching and popularization.

Of these alternatives, I personally prefer the third, but others may have a different preference.

This phenomenon of the multiple origin of a class has important bearings on general evolutionary theory that have been and will be considered elsewhere. It also involves some interesting morphogenetic problems, notably those of molar cusp homologies, that are beyond the scope of this review.

Literature Cited

Bohlin, B. 1945. The Jurassic mammals and the origin of the mammalian molar teeth. Bull. Geol. Inst. Uppsala, **31**: 363–388.

Broili, F., and J. Schroeder. 1936. Beobachtungen an Wirbeltieren der Karrooformation, XIX. Ein neuer Fund von *Tritylodon* Owen. Sitz.-ber. bayer. Akad. Wiss., **1936**: 187–228.

Broom, R. 1929. On some new light on the origin of mammals. Proc. Linn. Soc. New South Wales, **54**: 688–694.

Butler, P. M. 1939. The teeth of the Jurassic mammals. Proc. Linn. Soc. London, B, **109**: 329–356.

——. 1956. The skull of *Ictops* and the classification of the Insectivora. Proc. Zool. Soc. London, **126**: 453–481.

Crompton, A. W. 1958. The cranial morphology of a new genus and species of ictidosaurian. Proc. Zool. Soc. London, **130**: 183–216.

Huxley, J. S. 1958. Evolutionary processes and taxonomy with special reference to grades. Uppsala Univ. Arsskr., **1958**: 21–39.

Kermack, K. A., and F. Mussett. 1958a. The jaw articulation in Mesozoic mammals. XV Congr. Zool., Sect. V, Paper **8**: 1–2.

——. 1958b. The jaw articulation of the Docodonta and the classification of Mesozoic mammals. Proc. Roy. Soc., B, **148**: 204–215.

Kühne, W. G. 1949. On a triconodont tooth of a new pattern from a fissure-filling in South Glamorgan. Proc. Zool. Soc. London, **119**: 345–350.

——. 1950. A symmetrodont tooth from the Rhaeto-Lias. Nature, **166**: 696.

——. 1956. The Liassic therapsid *Oligokyphus.* British Museum (Natural History), London.

——. 1958. Rhaetische Triconodonten aus Glamorgan, ihre Stellung zwischen den Klassen Reptilia und Mammalia und ihre Bedeutung für die Reichart'sche Theorie. Pal. Zeitschr., **32**: 197–235.

Olson, E. C. 1944. Origin of mammals based upon the cranial morphology of therapsid suborders. Geol. Soc. Amer., Spec. Pap. **55**.

PARRINGTON, F. R. 1947. On a collection of Rhaetic mammalian teeth. Proc. Zool. Soc. London, **116**: 707–728.

PATTERSON, B. 1955. A symmetrodont from the early Cretaceous of northern Texas. Fieldiana, Zool., **37**: 689–693.

——. 1956. Early Cretaceous mammals and the evolution of mammalian molar teeth. Fieldiana, Geol., **13**: 1–105.

PEYER, B. 1956. Über Zähne von Haramyiden, von Triconodonten und von wahrscheinlich synapsiden Reptilien aus dem Rhät von Hallau, Kt. Schaffhausen, Schweiz. Schweiz. Pal. Abh., **72** [3rd paper]: 1–72.

SHIKAMA, T. 1947. *Teilhardosaurus* and *Endotherium*, new Jurassic Reptilia and Mammalia from the Jusin coal-field, south Manchuria. Proc. Japan Acad., **23**: 76–84.

SIMPSON, G. G. 1926. Mesozoic Mammalia. V. *Dromatherium* and *Microconodon*. Amer. Jour. Sci., **12**: 87–108.

——. 1928a. A catalogue of the Mesozoic Mammalia in the Geological Department of the British Museum. British Museum (Natural History), London.

——. 1928b. Some Triassic mammals. Amer. Jour. Sci., **15**: 154–167.

——. 1928c. The internal mandibular groove of Jurassic mammals. Amer. Jour. Sci., **15**: 461–470.

——. 1928d. Affinities of the Mongolian Cretaceous insectivores. Amer. Mus. Novitates, no. **330**.

——. 1929a. The dentition of *Ornithorhynchus* as evidence of its affinities. Amer. Mus. Novitates, no. **390**.

——. 1929b. American Mesozoic Mammalia. Mem. Peabody Mus. Yale Univ., **3**: Pt. 1.

——. 1937. Skull structure of the Multituberculata. Bull. Amer. Mus. Nat. Hist., **73**: 727–763.

ROMER, A. S. 1945. Vertebrate paleontology. Rev. ed. Univ. Chicago Press, Chicago.

YOUNG, C. C. 1940. Preliminary notes on the Mesozoic mammals of Lufeng, Yunnan. Bull. Geol. Soc. China, **20**: 93–111.

——. 1947. Mammal-like reptiles from Lufeng, Yunnan, China. Proc. Zool. Soc. London, **117**: 537–597.

NOTES AND COMMENTS

THE PERIANTH OF *ARISTOLOCHIA*—A NEW INTERPRETATION

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The peculiar perianth of *Aristolochia* has been repeatedly pointed out. Nevertheless, so far no one seems to have doubted the homology between this perianth and that of ordinary dicotyledons. Likewise, no doubt has been expressed on the homology between the trimerous, radially symmetrical perianth, typical for most members of the Aristolochiaceae, and the divergent bilaterally symmetrical perianth of the genus *Aristolochia*. Though several prominent botanists have dealt with this family, only Lotsy (1911) seems to have been suspicious, when he writes on the Aristolochiaceae "the whole group pushes me to bring them into relation with the Araceae, yet in what manner I do not know."

It is suggested that the bilaterally symmetrical perianth of Aristolochiae is not homologous with the radially symmetrical perianth of the Bragantieae and Sarumeae (Gregory, 1956), but has evolved from the foliage leaf of a lateral branch, to replace the original, radially symmetrical perianth, lost in the course of evolution. The "metamorphosis" from foliage leaf to perianth involved changes in colour and hairiness—including odoriferous glandular hairs—as well as the formation of a tubelike, rather solid portion, born on top of the inferior ovary, and enclosing the styles and the anthers, which together form the gynostemium.

The assumption that the original trimerous perianth was lost in *Aristolochia* seems justified in the light of the absence of petals in most genera of the Aristolochiaceae. The hypothesis of the homology between perianth and leaf is corroborated by the similarity in several species of *Aristolochia* (e.g. *A. maurorum*) between the size and contour of the foliage leaf and the cut-open perianth, as well as between their patterns of venation.

Striking support of the proposed interpretation derives from a shoot of *Aristolochia maurorum* recently found in Jerusalem (fig. 1). This shoot bears a series of teratological leaves which show a gradual acropetal transition from an almost perfect "perianth," without sex organs, to normal foliage leaves:

1) An almost perfect "perianth" with pedicel transformed to an almost closed groove, 7 mm. across.

2) A leaf with an almost completely metamorphosed lamina. One of the lateral lobes is concave as in the perianth; pedicel normal.

3) Leaves with abnormal spots which are similar to the normal perianth in colour, hairiness and anatomy.

The transformed pedicel is smooth on the outside, as well as thicker and more solid than the distal portion of the teratological leaf. In this it closely resembles the proximal part of the *Aristolochia* perianth, which forms a tube enveloping the sex-organs.

In *A. maurorum* and other species, flowers are born singly, at the basal nodes of lateral branches. The fact that the single flower is always opposite

FIG. 1. A lateral shoot of *Aristolochia maurorum* with teratological leaves (Jerusalem, March 1957).

the first leaf of the lateral branch fully accords with the view that the perianth is the metamorphosed first leaf of a lateral branch (*Aristolochia* has a ½ phyllotaxis).

The similarity between the spathe of *Arum* and other Araceae, which is clearly derived from a leaf (Engler, 1884), and the perianth of *Aristolochia* in both form, colour and biological role is another indication of the probable secondary nature of the *Aristolochia* perianth.

On the proposed theory, primitive species of *Aristolochia* are those in which, as in *A. maurorum*, the perianth agrees in form with an involute normal foliage leaf.

My thanks are due to Mrs. R. Koppel for preparation of the drawing.

Literature Cited

Engler, A. 1884. Ueber den Entwicklungsgang in der Familie der Araceen und ueber die Bluetenmorphologie derselben. Bot. Jahrb., **5**: 141–336.

Gregory, P. 1956. A phyletic rearrangement in the Aristolochiaceae. Am. J. Bot., **43**: 110–22.

Lotsy, J. P. 1911. Vortraege ueber botanische Stammesgeschichte III, Jena.

DATING THE ORIGIN OF LIFE ON EARTH

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With the development and increasing accuracy of radioactive dating methods for geological materials (Knopf, 1957) it has become apparent that our planet cannot have existed for more than about 4.5, or possibly 5 billions years. The close agreement of recent estimates (Russell and Allan, 1955; Patterson, Tilton, and Inghram, 1955) establishes the reasonable reliability of the methods.

It is well known, however, that fossil materials over about 0.5 billion years in age are very rare indeed. Considerable interest therefore centers on establishing a reasonably accurate date for the first appearance of life on Earth.

The processes by which life was produced from the non-living environment of the primitive earth, although as yet not fully understood, unquestionably took long periods of time to develop the dynamic physicochemical systems that would be universally recognized as "living." Calvin, 1956, has pointed out that before the simplest life-forms developed, there must have been a long period when organic compounds of gradually increasing complexity were being produced. These non-living biogenetic processes have been called "chemical evolution" and obviously merged with the better-known biological evolution. It is consequently impossible to claim that any particular stage of this continuous chain of complexity from simple organic compounds to multicellular organisms represents "life" while earlier stages do not; except by arbitary definition.

Nevertheless it is possible to approach the problem from the opposite direction. As far as is presently known, there is no method that can be used to detect the presence in the Earth's crust of the early organic compounds from which organisms arose. Indeed as Haldane, 1929, and Hardin, 1950, have pointed out, it is highly probable that this primary organic material was used up as food by the first organisms and none could have remained to form what might be termed "abiogenic fossils," i.e. fossilised traces of organic matter from non-living sources.

However a number of methods are available for the detection of simple fossil organisms. It should, therefore, be possible to establish at what period of the past simple forms of life were present on Earth. From the various published results it appears that the simplest organisms that are at present detectable in geological samples are certain types of bacteria and algae. It is very questionable whether any simpler systems can ever be shown to be present and consequently the age of these old fossil organisms can be taken as a minimal age for the origin of life.

The problem of exactly estimating the age of fossil samples is exceedingly complex and the difficulties further increase when the fossils are of very simple organisms. In fact the most important problem is often to first establish that the material under investigation is of biological origin and is not an inorganic artifact. Such difficulties must always be borne in mind when interpreting published results.

The oldest dated fossils so far reported are from Southern Rhodesian limestone (MacGregor, 1940) and have been dated by the age of the rocks in which they were found at about 2.7 billion years (Holmes, 1954; Ahrens, 1955). The fossils are of simple algae and, considering their probable age, are remarkably well preserved; so much so that it is difficult to question their biological origin.

The second oldest fossil materials to be re-

ported are from the Canadian shield. In this case, however, the rocks contained nothing visibly recognizable as the remains of organisms. Rankama, 1954, however, was able to show from a study of the isotopic carbon ratios of the samples of black slate that the C_{12}/C_{13} was similar to present-day organisms and different from inorganic carbon-containing minerals. The conclusion would seem to be that the Canadian rocks contained the remains of simple organisms whose form had been completely destroyed, but whose chemical constituents had become merged with the surrounding rocks. Estimates of the age of the slate samples, based upon the age of similar formations dated by radioactivity measurements, gave a value of approximately 2.55 billion years.

From other rocks of the Canadian shield it has proved possible to isolate samples of what appear to be fossil algae and bacteria (Tyler and Barghoorn, 1954). Again the appearance of the fossils leaves little doubt as to their biological origin. In this case the dating of the rocks was rather indirect and was based upon measurements of similar, previously aged formations. Nevertheless the estimated age of about 2 billion years is probably reasonably accurate.

No other fossil materials have been reliably dated as old as the above three examples and it must remain for more determinations to establish their general validity. Yet it is interesting and probably significant that the three ages are so similar.

Modern views of the origin of the Earth (Urey, 1952) lead to the conclusion that the primitive atmosphere was reducing and contained large amounts of hydrogen and hydrogen compounds. The changes in atmospheric composition that have produced the modern atmosphere are probably due to two main effects. Most of the lighter gases, and in particular hydrogen, must have escaped from the Earth by simple gravitational loss. While second, the release of free oxygen after the formation of widespread photosynthetic life, would lead to an accumulation of this gas.

This change from a reducing to an oxidizing atmosphere is largely due to the occurrence of life. Consequently estimates of the age of the oxidizing atmosphere can be taken as estimates of the age, and probably origin, of photosynthetic organisms. As many simple organisms are photosynthetic, it is probable that they were among the first forms of life.

From physical considerations involving estimates of the rate of loss of hydrogen molecules and ions from the Earth, Kuhn, 1956, has reached the conclusion that the oxygen atmosphere is about 2 billion years old. Similar estimates from entirely different measurements have also been published (Rankama, 1955). The nature of the gaseous components of the atmosphere must affect the oxidation state of various surface minerals and determinations of the ferrous/ferric ratio of old rocks give much higher values than recently formed rocks. This is additional proof that the Earth's primitive atmosphere was reducing. Dating of the rocks shows that a decrease of the ferrous/ferric ratio, presumably due to the release of free oxygen into the atmosphere by photosynthesis, began about 2 billion years ago.

Hence estimates of the age of atmospheric components make an interesting confirmation of the previously discussed ages of fossil organisms.

An alternative method for dating the origin of a specific group of simple organisms—the autotrophic sulfur bacteria—has been recently proposed (Thode, Macnamara, and Fleming, 1953). Studies of the ratio of sulfur isotopes S_{32}/S_{34} have shown that for modern sea-water sulfates the ratio is about 21.8, for cretaceous iron sulfides 23.0, and for pre-Cambrian sulfur-containing minerals 22.1. Detailed investigations of the ratios established that a change in sulfur isotopes began in rocks about 0.8 billion years ago. The workers suggest that this change is due to the presence of sulfur bacteria which must have originated about this time.

These results are most interesting, but are in apparent disagreement with all the previous estimates, particularly as it is suggested that the sulfur bacteria predate widespread photosynthetic life. In view of the great experimental difficulties involved in isotopic determination, it seems reasonable to wait for the work to be repeated before drawing any widespread conclusions. However it is possible that the sulfur bacteria are not such a primitive group of organisms as has been previously supposed.

The available evidence indicates that the earliest fossils, or traces of biological material, that are at present detectable, are in excess of 2 billion years old. Theoretical studies of the supposed primitive atmosphere of the Earth and of the oxidation state of iron in old rocks indicate that oxygen has been a component of the atmosphere for a similar period. It would therefore appear that although it is not possible to fix a date for the origin of life on Earth, complex chemical systems, such as algae and bacteria, apparently capable of photosynthesis, were present over 2 billion years ago.

Literature Cited

Ahrens, L. H. 1955. The oldest rocks exposed. Geol. Soc. Amer., Special paper, **62**: 155–168.

Calvin, M. 1956. Chemical evolution and the origins of life. Am. Scientist, **44**(**3**): 248–263.

Haldane, J. B. S. 1929. The origin of life. Rationalist Annual, **1929**: 3–10.

Hardin, G. 1950. Darwin and the heterotroph hypothesis. Sci. Monthly, **70**: 178–179.

HOLMES, A. 1950. The oldest dated minerals of the Rhodesian shield. Nature, **173**: 612–614.

KNOPF, A. 1957. Measuring geological time. Sci. Monthly, **85**: 225–236.

KUHN, W. 1956. The age of the oxygen atmosphere of the Earth. Chem. Ber., **89**: 303–308.

MACGREGOR, A. M. 1940. A pre-Cambrian algal limestone in Southern Rhodesia. Trans. Geol. Soc. South Africa, **43**: 9–16.

PATTERSON, C., G. TILTON, AND M. INGHRAM. 1955. The age of the earth. Science, **121**: 69–75.

RANKAMA, K. 1954. Early pre-Cambrian carbon of biogenic origin from the Canadian schield. Science, **119**: 506–507.

——. 1955. Geologic evidence of chemical composition of the Precambrian atmosphere. Geol. Soc. Amer., Special paper, **62**: 651–664.

RUSSELL, R. D., AND D. W. ALLAN. 1955. The age of the earth from lead isotope abundances. Mon. Not. Roy. Astro. Soc., Geophys. Suppl., **7**: 80–85.

THODE, H. G., J. MACNAMARA, AND H. W. FLEMING. 1953. Sulfur fractionation in nature and geological and biological time scales. Geochim. Cosmochim. Acta, **3**: 235–243.

TYLER, S. A., AND E. S. BARGHOORN. 1954. Occurrence of structurally preserved plants in the pre-Cambrian rocks of the Canadian shield. Science, **119**: 606–608.

UREY, H. C. 1952. The planets; their origin and development. New Haven, Conn.

LEPTURUS AND *MONERMA*: A REMARKABLE EXAMPLE OF PARALLEL DEVELOPMENT OF GROSS MORPHOLOGY IN GRASSES

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In a previous paper (in press) I pointed out the remarkable resemblance in the gross morphology between *Lepturus* and *Monerma*, in spite of the differences in the more minute cytological, anatomical and histological features between them. The striking morphological resemblance of these genera, accordingly, could be attributed to parallel (or convergent) evolution.

As described in the previous paper, the grouping of the members of *Lepturus* and *Monerma* into a single genus had been generally accepted until Hubbard's (1946) treatise on the taxonomy of these grasses was published. Hubbard enumerated seven morphological features as well as differences in a histological feature and in geographical distribution by which the two genera can be differentiated. Among these morphological features, one is concerned with the ligule, one with the lemma and the other five with the flower or caryopsis.

The shape of the ligule is similar in *Lepturus* and *Monerma*, but the ligule is ciliated in the former and glabrous in the latter. This character is of some systematic value, though not of importance. Various forms of ligules may be found within the same tribe. For example, some *Digitaria* species have glabrous ligules, while *Oplismenus*, a genus related to *Digitaria*, bears ciliated ligules, and in such genera as *Setaria*, *Pennisetum*, etc., belonging to Paniceae together with *Oplismenus* and *Digitaria*, the ligules are reduced to a fringe of short hairs. The lemmas are 3-nerved in both *Lepturus* and *Monerma*, but in the former the lateral nerves reach the apex, or extend nearly to it, and are glabrous or minutely hairy at the base, while in *Monerma* the lateral nerves are short and hairs are lacking. These differences, however, are not of any great systematic significance. In fact, there is variation in the length of lemma nerves even among members of the same genus. Also, the degree of hairiness on lemma often varies among the individuals of same species. Thus, although *Lepturus* and *Monerma* can be differentiated by these characters, they are very similar in the features of vegetative organs including glume, lemma and palea and also in the structure of spikelet. The following features are common in the two genera: spikelets solitary, sessile, narrow, sunk in the hollows of the hard, cylindric, articulate rachis; rachilla short, produced into a minute point, or bearing a rudimentary lemma; florets 1–2, bisexual; glumes of the terminal spikelet equal and similar, the lower ones minute or suppressed in the lateral spikelets while the upper ones close the cavity of the rachis and are coriaceous, rigid, narrow, acute or acuminate, nerved; lemmas shorter than the glumes, 3-nerved, hyaline, lanceolate to oblong; palea hyaline, a little shorter than, or nearly equal to, the lemma. The features of glume, lemma, spikelet structure and inflorescence are of great value in the traditional systems of grasses. It is thence natural that the members of *Lepturus* and *Monerma* have been treated as belonging to the same genus until Hubbard's paper and its

TABLE 1. *Distinctive features of flower and caryopsis in* Lepturus *and* Monerma *(after Hubbard, 1946)*

Lepturus	*Monerma*
1. Lodicules oblong or obcuneate.	Lodicules lanceolate to ovate.
2. Style slender, distinct.	Style extremely short or obsolete.
3. Grain tipped by the persistent style bases.	Grain tipped by a shrivelled brown truncate appendage.
4. Scutellum elliptic, half to two-thirds the length of the grain.	Scutellum broadly elliptic, about one-fifth the length of the grain.
5. Hilum basal, orbicular.	Hilum linear-oblong, slightly above the base of the grain.

support on anatomical and histological grounds by Hansen and Potztal (1954).

Turning to the features of flower and caryopsis, we find another situation. Table 1 lists the distinctions between *Lepturus* and *Monerma* in flower and caryopsis characters. Based on the features of anatomy, histology and cytology, Pilger (1954) and I (Tateoka, 1957) have referred *Monerma* to Pooideae and *Lepturus* to Eragrostoideae. I propose to consider the five morphological features indicated in table 1 in relation to this question:

1) Oblong or obcuneate lodicules are common in Eragrostoideae, but not found in Pooideae (sens. Tateoka, 1957). Pooideae are characterized by the lodicules being lanceolate, ovate, oval and acute, acuminate or split in two, symmetric or asymmetric, acute or acuminate halves. The lodicules of *Lepturus* clearly show the features of Eragrostoideae and those of *Monerma* the characteristics of Pooideae.

2) Distinct styles, as found in *Lepturus*, are shared by Pooideae and Eragrostoideae. But, a number of species of Pooideae have the styles extremely short or obsolete as in *Monerma*, while such styles seem very seldom in Eragrostoideae. With respect to this character, *Monerma* is related to Pooideae and *Lepturus* is similar to the large majority of Eragrostoideae.

3) Grains tipped by a shrivelled truncate appendage as occurring in *Monerma* are not so rare in Pooideae, and uncommon in Eragrostoideae. Grains tipped by the persistent style base as in *Lepturus* are found in many species of Eragrostoideae and in some Pooideae.

4) The relative size of embryo to endosperm shows a difference between Pooideae on the one hand and Eragrostoideae and Panicoideae on the other. In the former the embryos are generally small, the grain consisting largely of endosperm, while in the latter the embryos are relatively large, and the proportion of endosperm is correspondingly reduced (Reeder, 1957). *Lepturus* has features similar to Eragrostoideae and Panicoideae, and *Monerma* resembles Pooideae.

5) The features of the hilum are of some systematic value, and each genus shows considerable uniformity in this respect. But, the differences—orbicular vs. linear, and also basal vs. slightly above the base—vary within a tribe. For example, among Festuceae, *Festuca*, *Lolium*, etc. have linear hilums and in *Dactylis*, *Puccinellia*, *Poa*, etc. hilums are of punctiform. This character thus seems useless in differentiating Pooideae from Eragrostoideae.

The characteristics of flower and caryopsis of *Lepturus* imply various indications of the close relationship of the genus to the typical members of Eragrostoideae. The same is true of *Monerma* relative to Pooideae. The relationship of *Lepturus* to Eragrostoideae and that of *Monerma* to Pooideae are reflected in morphology, even though not in features customarily considered as important in the traditional systematics. However, since morphological features of all groups of living organisms seem to have a phylogenetic background, it may seem unlikely that *Monerma* has evolved from Pooideae, whereas *Lepturus* which has morphological features very close to *Monerma* in the characters of inflorescence, spikelet structure, glume, lemma, etc. should have been derived from Eragrostoideae. Still, if one considers the fact that members of both groups, Pooideae and Eragrostoideae, are much diversified in morphological characteristics, *Lepturus* and *Monerma* belonging to different groups may share some morphological features. Thus, it seems obvious that the features of inflorescence, glume, lemma, etc. should not be taken in systematics in the sense used in the traditional systems, and also that in the classification of grasses, stress should be laid on the characteristics of the flower itself and caryopsis. Stebbins (1956a,b) points out the importance of lodicule and caryopsis in grass systematics. Church (1949), somewhat earlier, recognized the distinction of *Torreyochloa* from *Glyceria* in the features of chromosomes, leaf epidermis, lodicule and caryopsis. The parallel evolution of gross morphology in *Lepturus* and *Monerma* illustrates the danger of treatment of morphological characters according to the traditional system. Moreover, it shows that in grass systematics not only morphological features, such as glume, lemma, sterile floret, etc., but the characters of the flower proper and caryopsis should be subjected to thorough examination.

LITERATURE CITED

CHURCH, G. L. 1949. A cytotaxonomic study of *Glyceria* and *Puccinellia*. Amer. Jour. Bot., **36**: 155–165.

HANSEN, I., AND E. POTZTAL. 1954. Beitrage zur Anatomie und Systematik der Leptureae. Bot. Jb., **76**: 251–270.

HUBBARD, C. E. 1946. *Henrardia*, a new genus of the Gramineae. Blumea, Suppl. 3: 10–21.

PILGER, R. 1954. Das System der Gramineae. Bot. Jb., **76**: 281–384.

REEDER, J. R. 1957. The embryo in grass systematics. Amer. Jour. Bot., **44**: 756–768.

STEBBINS, G. L. 1956a. Taxonomy and the evolution of genera, with special reference to the family Gramineae. Evolution, **10**: 235–245.

——. 1956b. Cytogenetics and evolution of the grass family. Amer. Jour. Bot., **43**: 890–905.

TATEOKA, T. 1957. Miscellaneous papers on the phylogeny of Poaceae (10). Proposition of a new phylogenetic system of Poaceae. Jour. Jap. Bot., **32**: 275–287.

——. In press. Notes on some grasses. VII. Cytological evidence for the phylogenetic difference between *Lepturus* and *Monerma*. Cytologia.

COMMENTS ON QUANTITATIVE SYSTEMATICS[1]

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In a recent issue of this Journal Dr. R. F. Inger (1958) has published an original and stimulating contribution concerning the definition of a genus. I regret, therefore, that much of the paper is concerned not with an elaboration of his views, but with an attack upon methods of quantifying systematic relationships developed by Prof. C. D. Michener and myself (Michener and Sokal, 1957). My regrets are the more profound since I fail to see that the views expressed by us and those of Dr. Inger are necessarily mutually exclusive and that the establishment of his views necessitates the disproof of ours. While disinclined to polemical debate I yet feel it incumbent upon me, particularly in the absence from this country of Prof. Michener, to reply to some of the criticism leveled at us by Dr. Inger and to dispel a number of erroneous impressions conveyed to readers of his paper.

If I read him correctly, Inger bases his criticism of our method on three main grounds:

(1) He disputes the relative objectivity of the choice of characters in our study of 97 species of the *Hoplitis* complex of bees. Three specific instances are cited by Inger in refutation of our claims of objectivity:

(a) ". . . the selection of characters, even if one choses 122 of them, is also subjective." Selection is an inappropriate term to apply to our procedure. We did not select the characters in the sense of taking some and rejecting others. We took all the characters that were known for these species, leaving out only those that were clearly redundant or those that were constant for the entire group. It is true that we could have attempted to find more characters. This would have meant engaging in a renewed systematic study of the genera in search for new characters. Any scientific study has selected data in the sense that a limited amount of information is used and a judgement based upon it. In almost every study more information could have been obtained by more research. Yet this fact by itself is not grounds on which to impugn the objectivity of the research.

Another point which might easily not be appreciated by persons unacquainted with multivariate analysis is the virtual impossibility of introducing deliberate bias into a 97×97 matrix based on 122 items. The data are so numerous and the computations so complex that manipulation of the data in this manner would be considerably more difficult, if at all feasible, than the search for additional characters.

(b) Subjectivity is claimed by Dr. Inger for our "assigning of numerical values to different states of non-meristic characters." This procedure is illustrated by our treatment of continuously varying ratios in which classes with arbitrary limits were established and identified with code numbers used in computation. The use of class marks with discrete code numbers for continuous variables is standard procedure in statistics as any elementary text book on biometric techniques will show.

(c) A third supposedly non-objective procedure is the arbitrary placing of species exhibiting borderline phenotypes into one or the other of two adjoining classes. Again, this is an indictment of a standard operating procedure in statistics. The only really significant point, however, is not the arbitrariness of the placing of a given species in one class or the other, but the well-known negligible effect of such placement on the magnitude of the correlation coefficient,

[1] Contribution No. 1036 from the Department of Entomology, University of Kansas, Lawrence.

which after all is the only measure of relationship employed in our study. Unless a serious distortion of the correlation coefficients can be demonstrated I cannot see any cause for concern. Perusal of the technical part of our study (Sokal and Michener, 1958) may dispel Inger's doubts on this matter.

In addition to the specific refutations of Inger's charges of subjectivity I should add that subjectivity and objectivity as applied to the problem under consideration cannot be contrasted as absolutes but are relative concepts. Objectivity may be considered as an ideal goal which can probably never be achieved but only approached. If by objectivity in choice of characters we mean the inclusion of all known characters in our study without regard to the effect such inclusion will have on the eventual classification of the group then we can clearly state that this has been our aim. Whether it has been fully realized is left for the reader to judge.

(2) Dr. Inger disapproves of our failure to weight characters, or rather our equal weighting of all characters. The discussion of this point in our paper is quite complete and need be restated here only in summary form: Weighting is dangerous since it is usually done in ignorance of the underlying genetic basis of a given character and also since we do not know much about the genetic correlations among characters in customary taxonomic work. Believing in the general hypothesis that most genes will affect many characters and conversely that every character will reflect the effects of many genes, we feel that a random selection of characters will result in a random sample of the genotype of the organism. We believe furthermore, that on choosing a large number of characters we can observe proportionally more of the genotype. In quoting us to the extent that "(we) believe that weighting becomes unnecessary" Dr. Inger omitted our reasons for this statement and added that no mathematical or other proof has been given for it. However, this quotation would have been improved if the rest of the sentence from which it had been taken had been added, namely, that weighting becomes unnecessary because the magnitude of correlation coefficients calculated between species would be little affected, except by extreme weighting.

After setting up three premises necessary in his opinion for the equal weighting of characters, Dr. Inger proceeds to show that the first two premises are "demonstrably false." I am not prepared to argue the merits of these premises in detail since I do not believe them to be pertinent to our own argument. It is, however, unfortunate that Dr. Inger chose to illustrate his point with material leading to far from unequivocal conclusions. The genetics and evolution of dentition and coat color in mammals are contrasted. Dentition is said to be relatively resistant to evolutionary modification since it is subject to greater checks, while coat color, being of less significance, appears to be under weaker control of natural selection and subject to strong modification by single loci. Furthermore, changes in the dentition are said to have correlated effects on other parts of the animal, while "a change in coat color has no direct effect on other structures."

It is, however, a well known fact that single genes in mammals can bring about marked changes in the animals' dentition (see Grüneberg, 1952 for several examples in the mouse). Furthermore pleiotropic effects of coat color or pigmentation in mammals have been observed by a number of authors, notably MacArthur (1944) in mice, Haldane (1954) in man, etc. The microphtalmia gene in mice affects both pigmentation and teeth (Grüneberg, 1952). Owing to the fundamental biochemical processes involved in pigment formation it would appear surprising if changes in coat color were not correlated with other changes in the animals' morphology and physiology.

In view of the many cases of adaptive coloration which appear to exist in mammals it seems dubious that coat color is under less environmental control than dentition. This is certainly not a point on which definitive opinions can be expressed in view of our present knowledge. To say, therefore, that one of these characters has greater weight than another (unless such a statement is backed up by phylogenetic considerations, which would almost necessitate a fossil record) is the very kind of extrapolation beyond the data which our paper was trying to prevent.

This is not to say that dentition may not be a superior indicator of taxonomic relationships in mammals when customary taxonomic methods are employed. However, when a *Q*-type correlation study based on a great number of variables is undertaken our hope would be that more useful information can be extracted from the totality of the phenotype than from any one character, however diagnostic it may be. Should dentition really be such an important and critical character this should be reflected in a great many other correlated characters and weighting should thus be essentially automatic by our method.

When the problem of character evaluation is seen from the viewpoint of all phyla of plants and animals rather than from the parochial perspective of vertebrate systematics the idea of assigning adaptive or genetic evaluations becomes entirely quixotic. Who would venture to evaluate the adaptive significance of a tibial spur compared with that of punctate elytra in an insect?

We are supported in our views by the independently reached conclusions of Sneath (1957) who in a different group of organisms felt again that the only logically defensible method of

classification was the employment of many but unweighted characters.

(3) The use of static descriptive relationships (i.e. relationships based on degrees of difference between organisms) is condemned as "typological" and we are told that "all taxonomic categories must be in harmony with evolutionary concepts." This is a goal in which I heartily concur, but as pointed out by Michener (1957), it is often impossible with current techniques to produce a natural classification combining both static and phylogenetic relationships. It is only natural that once static relationships are determined, phylogenetic speculation upon these results will follow. Not only does our method not prevent this, but we heartily endorse it, and in the latter part of our paper have attempted phylogenetic diagrams modified from those of static relationships. We only maintain that initially a system of classification should attempt to do only one or the other, namely trace phylogeny or trace static relationships and that the description of these relationships be done in an objective manner. The reader is referred to a detailed discussion of this point by Michener (1957).

I fail to understand in this connection the criticism of our assumption that the greater the phenotypic resemblance the greater the genetic resemblance. It is still the underlying assumption of genetics that like begets like. I cannot agree with Inger that such views are Linnaean, since Linnaeus' natural classification was classification *per se* without thought of any genetic relationship.

Dr. Inger made the point that biological entities require "biological" methods of classification and that purely mechanical means of classification cannot be applied to such data. Classification is an abstract philosophical concept which can be applied equally to living as well as non-living objects, to ecological communities as well as to biological systematic categories. At the University of Kansas a routine introduction to the use of taxonomic keys in beginning entomology classes is the keying out of a collection of different types of screws involving flatheads, roundheads, long and short stems and different types of threads. There is little question that a natural classification of screws can be based on a series of such characteristics. Inger's point is that such a classification is purely static and could not possess a historical dimension. However, a careful study of the types of screws in the collection might reveal evidences of the method of manufacture and might lead to correct conclusions regarding the types of machines by which they had been made, the processes of manufacture they had undergone and the types of situations for which the screws would be adapted. A comparative study of "fossil screws," i.e. earlier models, might reveal "evolution" of design, manufacturing methods and materials. The analogies with biological evolution are real and applicable.

I do not wish to prolong this discussion except to comment briefly on two points of Dr. Inger's alternative procedure, namely the use of the adaptive approach: (1) for each genus that is clearly ecologically defined another one could easily be found showing ecological diversification. It could be argued hence that such a taxon does not represent a genus. However, the abolition and splitting of genera on this basis would surely engender more confusion than illumination. Moreover, using the adaptive approach what are we to do with infra- and supra-generic categories? (2) The weighted variable group method proposed by Michener and Sokal tried to evaluate quantitatively the relations between taxonomic units, which for our pilot study were species. It was not devised for the purpose of defining genera although it can serve for that purpose if one bases a classification on static relationships. Hence my statement at the beginning that I do not feel that our two aims are necessarily conflicting.

I would not like to leave the reader with the impression that Michener and I consider our methods beyond reproach or criticism. On the contrary; we are fully aware that ours is a pilot study and we expected criticism to be leveled against it on the basis of the choice of number of states, the choice of the correlation coefficient, the relatively arbitrary promulgation of ex-group versus pre-group species, and a number of other weaknesses in our procedure, several of which were pointed out in the original paper. Our only defense in this matter is that we had to limit our aims somewhat in this first study and that we are making current attempts to improve our procedures. I cannot, however, in all honesty see the pertinence of Dr. Inger's published criticism.

Postscript—Readers of EVOLUTION may be interested to learn that programs have been prepared for the IBM 650 digital computer (with automatic indexing accumulators and floating decimal arithmetic), which carry out an analysis essentially identical to the weighted variable group method in a matter of a very few minutes. Inquiries regarding the use of these programs, entitled Taxon I and II, should be addressed to the author.

LITERATURE CITED

GRÜNEBERG, H. 1952. The genetics of the mouse. Martinus Nijhoff, The Hague. 650 pp.

HALDANE, J. B. S. 1954. The biochemistry of genetics. George Allen & Unwin, Ltd., London. 144 pp.

INGER, R. F. 1958. Comments on the definition of genera. Evolution, **12**: 370–384.

MACARTHUR, J. W. 1944. Genetics of body size and related characters. II. Satellite characters associated with body size in mice. Amer. Nat., **78**: 224–237.

MICHENER, C. D. 1957. Some bases for higher categories in classification. Syst. Zool., **4**: 160–173.

——, AND R. R. SOKAL. 1957. A quantitative approach to a problem in classification. Evolution, **11**: 130–162.

SNEATH, P. H. A. 1957. Some thoughts on bacterial classification. Jour. Gen. Microbiology, **17**: 184–200.

SOKAL, R. R., AND C. D. MICHENER. 1958. A statistical method for evaluating systematic relationships. Univ. Kansas. Sci. Bull., **38**: 1409–1438.

REPLY TO SOKAL

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If scientists were not human, the progress of science would be smoother and more rapid. But let us face reality: Dr. Sokal and I could continue this argument endlessly, and probably much of what we would say would not enlighten our colleagues or advance our science. Time will tell if either party to this little dispute is correct and to what extent. That being so, I will restrict my comments to one point though I disagree with much that Sokal writes in his note and believe that he has failed to understand my argument probably as much as I have misunderstood his.

In their original contribution, Michener and Sokal criticized conventional systematic methods, in part because of the subjective element in those methods. One of the advantages of the quantitative method they proposed was, according to them, its objectivity. The words "subjective" and "objective" were not qualified as being relative. I am gratified to see that Dr. Sokal has now made this qualification.

Contrary to Dr. Sokal's statement that one of my basic criticisms of his and Dr. Michener's work is its departure from objectivity, it was my purpose in that portion of my paper to show that their method suffered from the same limitation as "conventional" taxonomic methods (whatever they are). It is important to recognize that manipulation of data or numbers by any procedure does not increase the objectivity of the process by which those data or numbers were obtained. Interpretation of data are made in the mind and in that sense are subjective. A discipline that merely accumulates facts is not a science. Systematic methods, therefore, will always be subjective to some extent.

BOOKS RECEIVED

Notices will be given under this heading of books of evolutionary interest sent to the Editors. Appearance of a title in this section will not preclude a fuller review in a later issue of the journal.

Glass, Bentley, Owsei Temkin, William L. Straus Jr. (editors) 1959. Forerunners of Darwin: 1745–1859. 471 pp. Illus. $6.50. Johns Hopkins Press. A collection of fifteen historical essays by seven authors largely based on contributions to The History of Ideas Club at Johns Hopkins University.